44714

National Library of Canada
Canadian Theses Division
Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Division des thèses canadiennes

PERMISSION TO MICROFILM — AUTORISATION DE MICROFILMER

• Please print or type — Écrire en lettres moulées ou dactylographier

Full Name of Author — Nom complet de l'auteur

PAUL DUNCAN COOPER

Date of Birth — Date de naissance	Country of Birth — Lieu de naissance
20 DEC 1942	CANADA

Permanent Address — Résidence fixe

700 LEE RIDGE ROAD
EDMONTON, ALBERTA
T6R 0P2

Title of Thesis — Titre de la thèse

TRANSPORT OF OPTICALLY DETECTABLE ORGANELLES IN NORMAL AND INTERRUPTED MYELINATED NERVE FIBERS.

University — Université

UNIVERSITY OF ALBERTA

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée

Ph. D.

Year this degree conferred — Année d'obtention de ce grade	Name of Supervisor — Nom du directeur de thèse
1980	DR. RICHARD S. SMITH

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

Date	Signature
23 APRIL 1980	Paul D. Cooper

NL-91 (4/77)

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

Ottawa, Canada
K1A 0N4

NL-339 (Rev. 8/80)

THE UNIVERSITY OF ALBERTA

TRANSPORT OF OPTICALLY DETECTABLE ORGANELLES IN NORMAL AND INTERRUPTED MYELINATED NERVE FIBERS

by

© PAUL D. COOPER

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

EDMONTON, ALBERTA

SPRING, 1980

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled TRANSPORT OF OPTICALLY DETECTABLE ORGANELLES IN NORMAL AND INTERRUPTED MYELINATED NERVE FIBERS submitted by PAUL D. COOPER in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

R. S. Smith

Supervisor

G. S. Grend

[illegible]

[illegible]

E. J. Sundeen ... H. Pearse

Mat A. Bray

External Examiner

Date 31 March 1980

University of Alberta

DEDICATION

TO MY PARENTS AND WIFE,

IN APPRECIATION OF THEIR SUPPORT

ABSTRACT

The axonal transport of optically detectable organelles was investigated in segments of intact, damaged, and regenerating axons from the amphibian Xenopus laevis.

In lengths of axon isolated from intact nerves, three groups of moving organelles were distinguished. Particles with round or slightly elliptical images about 0.2-0.5 μm in diameter moved in the direction of the cell body at an average velocity of about 1 μm/sec. A similarly shaped group of organelles moved in the opposite direction at approximately the same average velocity. The movement of organelles in both of these groups was saltatory and except for intermittent hesitations and reversals of a few micrometers, each particle continued to move in the same direction, independently of the others. The somatopetally moving group contained about ten times as many detectable organelles as did the somatofugally moving group. The third group consisted of rod-shaped bodies approximately 0.2-0.3 μm in diameter with lengths ranging from about 1-8 μm. These usually remained stationary in the axoplasm, but occasionally exhibited axial movement in either direction similar to that of the round organelles.

The sources of variability in measures of particle velocity and the amount of particle traffic were

investigated for somatopetally moving particles in normal animals. For both particle velocity and the numbers of particles crossing a given diameter of axon the major source of variation was between individual measurements in individual axons. A statistically significant variation was found between axons but not between animals.

In crushed and regenerating axons examined at periods from 1 to 128 days after crushing, no change was found in the velocity of particles travelling either somatopetally or somatofugally. However the amount of detectable particulate material transported did change. Somatopetal particle traffic 1 cm proximal to the crush, while not eliminated, was significantly depressed at 1 and 2 days ($P<0.001$) and at 4 days ($P<0.05$). No significant departure from normal levels and observed at 8, 16 and 34 days, but a significant increase ($P<0.01$) to almost double the normal values occurred at 64, 69 and 100 days. Somatofugal particle traffic was elevated at 8, 64 and 69 days after the crush, and reduced at 100 days ($P<0.05$). Other differences were also found in the regenerating axons. Increases in the numbers of rod-shaped organelles moving somatopetally and somatofugally were detected. An increase occurred in the average diameter of the images of the somatopetally moving particles; the maximum image diameter increased from 0.7 to 1.1 μm. Examination of a large number of axonal cross sections by electron microscopy revealed the occasional presence in the regenerating axons of unusually large

organelles with a lamellar membranous appearance.

Two aspects of particle behavior near axonal interruptions were analyzed to investigate the origin of the somatofugally travelling organelles observed at short time periods in axons proximal to the crush. It was found that rapid and extensive degeneration of the axoplasmic structure was caused by certain components of amphibian Ringer solution, particularly calcium and sodium ions. However, the effects of ions on axonal structure and on particle transport are separable, since transport was not maintained in solutions which eliminated the gross structural changes. Evidence was also obtained which suggested that transported particles may undergo a reversal of direction at the site of an axonal interruption. It was concluded that at both early and later stages of regeneration, the amount of somatopetal particle transport is related primarily to the changing conditions at the distal ends of the axons.

ACKNOWLEDGEMENTS

I wish to express a particular debt of gratitude to Dr. Richard S. Smith and to Dr. Dirk L. Schaeffer, whose personal interest and generous assistance will long be appreciated.

I also thank Mrs. Sita Prasad, Mrs. Edith Fearon, Judi Gould and Mrs. Louiza Gordon for their valuable help at various stages of preparation of this thesis, and Dr. G. Monckton for the use of the Muttart-Collip Electron Microscope Laboratory.

This work was supported in part by a pre-doctoral fellowship from the Muscular Dystrophy Association of Canada.

Table of Contents

Chapter Page

CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW.....1

INTRODUCTION.....1

EARLY SPECULATIONS AND OBSERVATIONS.....2

COMPRESSION AND CONSTRICTION EXPERIMENTS.....6

LONG TERM EFFECTS OF PARTIAL CONSTRICTIONS....6

SHORT TERM ACCUMULATIONS AT COMPLETE CONSTRICTIONS.....8

Specified Enzymes.....9

Neurotransmitters and Neurosecretory Substances.....11

Interpretation of Accumulations at Constrictions.....13

TRANSPORT OF RADIOACTIVELY LABELLED TRACERS.....17

SLOW TRANSPORT.....18

TRANSPORT AT HIGHER VELOCITIES.....19

CHARACTERISTICS OF THE TECHNIQUE.....21

SOMATOPETAL TRANSPORT.....22

CENTRIPETAL SPREAD OF NEUROTROPIC VIRUSES AND BACTERIAL TOXINS.....22

EVIDENCE FROM CONSTRICTION STUDIES.....24

MICROSCOPIC OBSERVATION OF ORGANELLE MOVEMENTS IN AXONS.....26

RADIOACTIVE LABELLING OF SOMATOPETALLY MOVING MOLECULES.....29

SOMATOPETAL TRANSPORT OF NON-RADIOACTIVE TRACERS.....31

SELECTIVITY OF SOMATOPETAL TRANSPORT.....33

COMMENT.....35

AXONAL TRANSPORT DURING DEGENERATION AND REGENERATION....36

TRANSPORT IN DISTAL PORTIONS OF DAMAGED AXONS....36

SIGNIFICANCE OF AXONAL TRANSPORT IN REGENERATING NEURONS....38

SOMATOFUGAL TRANSPORT IN PROXIMAL PORTIONS OF DAMAGED AXONS....40

Attempts to Relate Axonal Transport to Regeneration....40

Problems of Interpretation and Experimentation....44

DIFFICULTIES IN IDENTIFYING GENERAL RESPONSES DURING REGENERATION....46

COMPARISON WITH TRANSPORT DURING EMBRYONIC AND POSTNATAL DEVELOPMENT....50

SOMATOPETAL TRANSPORT IN PROXIMAL PORTIONS OF DAMAGED AXONS....55

AXONAL TRANSPORT IN REGENERATING SPROUTS....59

STATEMENT OF OBJECTIVES....60

CHAPTER 2 OPTICAL DETECTION OF ORGANELLE TRANSPORT IN NORMAL AXONS....61

INTRODUCTION....61

METHODS....61

RESULTS....70

GENERAL OBSERVATIONS....70

STATUS OF THE PREPARATIONS....72

MOTION OF THE ROUND ORGANELLES....73

Direction of Motion....73

Velocities....74

Motion across Nodes of Ranview....82

MOTION OF THE ROD-SHAPED ORGANELLES....85

RELATIONSHIP BETWEEN THE MOTION OF ROUND ORGANELLES AND THE RODS.....87

DISCUSSION.....89

PARTICLE TRANSPORT IN EXCISED SEGMENTS OF MATURE NERVES.....89

DIRECTIONS AND VELOCITIES OF ORGANELLE MOVEMENTS.....93

NATURE OF THE OPTICALLY DETECTABLE ORGANELLES.....98

MECHANISMS UNDERLYING THE MOVEMENT.....101

CHAPTER 3 VARIABILITY IN ORGANELLE TRANSPORT.....102

INTRODUCTION.....102

METHODS.....103

RESULTS.....105

PARTICLE VELOCITIES.....105

NUMBERS OF PARTICLES.....111

CORRELATIONS BETWEEN VARIABLES.....114

DISCUSSION.....116

SOURCES OF VARIABILITY.....116

VARIATION ASSOCIATED WITH INDIVIDUAL PARTICLES.....116

VARIATION BETWEEN AXONS.....118

VARIATION BETWEEN ANIMALS.....121

CORRELATIONS BETWEEN VARIABLES.....122

CHAPTER 4 ORGANELLE TRANSPORT IN LOCALLY CRUSHED AXONS AT TIMES OF ONE DAY AND LONGER AFTER THE CRUSH.....125

INTRODUCTION.....125

METHODS.....126

RESULTS.....129

ASSESSMENT OF REGENERATION.....129

GENERAL OBSERVATIONS ON PARTICLE TRANSPORT..130

ORGANELLE TRANSPORT PROXIMAL TO THE LESION..131

Numbers of Particles Transported.....131

Numbers of Rod-shaped Organelles in Motion.....136

The Size of Particulate Images.....137

ORGANELLE TRANSPORT DISTAL TO THE LESION....139

DISCUSSION.....143

THE INTERPRETATION OF REGENERATION EXPERIMENTS.....143

DEMONSTRABLE CHANGES IN PARTICLE TRANSPORT DURING REGENERATION.....145

CONTINUED BIDIRECTIONAL TRANSPORT IN CRUSHED AXONS.....147

CHAPTER 5 LOCAL REACTIONS AT SITES OF DAMAGE IN SINGLE AXONS.....151

INTRODUCTION.....151

METHODS.....152

RESULTS.....154

ORGANELLE TRANSPORT IN COMPRESSED AXONS.....154

ORGANELLE TRANSPORT IN CRUSHED AXONS.....157

Experiments in Ringer Solution.....157

Experiments with Modified Solutions.....163

Summary of the Effects of Ions Present in the Bathing Solutions.....171

REVERSAL OF DIRECTION OF ORGANELLE TRANSPORT AT A MINIMAL LESION.....173

DISCUSSION.....179

LOCAL EFFECTS OF EXTRACELLULAR IONS ON DAMAGED AXONS.....179

Gross Structural Changes.....179

Arrested Particle Transport.....180

REVERSAL OF DIRECTION OF AXONAL TRANSPORT AT AXONAL INTERRUPTIONS.....181

CHAPTER 6 GENERAL DISCUSSION AND CONCLUSIONS.....185

STUDY OF ORGANELLE TRANSPORT BY LIGHT MICROSCOPY.....185

SUMMARY OF CHANGES IN PARTICLE TRANSPORT IN RELATION TO CONDITIONS AT CRUSHED ENDS.....190

CONTINUATION OF TRANSPORT AFTER INTERRUPTION OF NORMAL SUPPLY.....196

Particles from the Unaffected Region.....196

Particles from the Stationary Region.....197

Reversal of Somatofugally Moving Particles..198

Injury-generated Particulate Material.....198

Material Originating Externally.....199

REFERENCES.....201

LIST OF TABLES

Table		Page
3.1	Analysis of variance summary table for average velocities of somatopetally moving particles in amphibian sciatic nerve fibers	109
3.2	Analysis of variance summary table for numbers of somatopetally moving particles per minute in amphibian sciatic nerve fibers	115
5.1	Prograssive change in relative numbers of proximally and distally moving particles on distal side of region of reversal	172
5.2	Number of particles entering and leaving each side of region of reversal	176
5.3	Effects of applied solutions on axonal structure and transport near crush injuries	178

LIST OF FIGURES

Figure		Page
2.1	Diagram of the dissecting and viewing chamber	63
2.2	Diagram showing predominant directions of particle movements in the 8th spinal roots and the sciatic nerve	75
2.3	Histogram showing the numbers of somatopetally and somatofugally moving particles crossing an axon diameter in alternate 1 minute intervals	76
2.4	Characteristics of the movement of individual particles in the somatopetal and somatofugal directions	78
2.5	Distance-time plots for particles travelling somatopetally and somatofugally	80
2.6	Histograms of average velocities of individual particles travelling in the somatopetal and somatofugal directions in sciatic nerve fibers.	81
2.7	Trajectories of particles crossing a node of Ranvier in the somatopetal direction	83
2.8	Distance-time plots for particles crossing a node of Ranvier in the somatopetal direction	84
2.9	Distance-time plot for a rod-shaped organelle travelling in the somatofugal direction	86
2.10	Diagram illustrating an observed relationship between the movements of a round and a rod-shaped organelle	88
3.1	Distribution of average velocities of 1,100 somatopetally moving particles in amphibian sciatic nerve fibers	106

3.2 Comparison of mean velocities and 95% confidence intervals for somatofugally moving particles between different axons and animals.....107

3.3 Relationship between distribution of average velocities, mean velocity and standard deviation of somatofugally moving particles in 3 axons.....110

3.4 Relationship between mean velocity and standard deviation of somatofugally moving particles in 22 axons.....112

3.5 Comparison of mean times between particles and 95% confidence intervals for somatofugally moving particles between different axons and animals.....113

4.1 Mean somatopetal and somatofugal particle velocities in normal and regenerating amphibian axons.....132

4.2 Numbers of somatopetally and somatofugal moving particles per minute in normal axons and in axons proximal to crushes applied 1-100 days previously.....133

4.3 Relative changes in mean numbers of somatopetally and somatofugally travelling particles on the proximal side of axonal crushes.....135

4.4 Proportion of somatopetally moving particles at successive regeneration periods with image diameters beyond the range observed in normal axons.....138

4.5 Numbers of somatopetally and somatofugally moving particles per hour in distal portions of axons as a function of time after crush.....142

4.6 Predicted decline in numbers of somatopetally moving particles per minute at specified distances proximal to axonal interruption.....149

5.1 Diagrammatic representation of sequential stages of degeneration adjacent to axonal crushes.....160

5.2 Progress of respective stages of degeneration on proximal and distal sides of crush in Ringer solution.....162

5.3 Distal progress of respective stages of degeneration in isotonic potassium chloride.....164

5.4 Effect of calcium on development of degenerative changes.....166

5.5 Extent of fragmentation of elongated organelles in different solutions.....168

5.6 Diagram of region of axon where reversal of particle direction was observed.....174

6.1 Relation of numbers of transported particles to changing conditions in crushed axon.....191

LIST OF PLATES

Plate		Page
2.1	Darkfield photomicrographs showing the appearance of organelles within the axoplasm of a myelinated sciatic nerve fiber from *Xenopus laevis*	67
2.2	Photographs of sequential frames from 16 mm motion picture records showing stationary and moving particles	69
4.1	Electron micrograph of large, dense lamellar body (DLB) in axon examined 14 days after crushing	141
5.1	Darkfield photomicrographs showing material appearing at local axonal compressions	156
5.2	Darkfield photomicrographs of degenerative transformations adjacent to axonal crushes	159

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Knowledge of the process by which materials are moved axially inside nerve fibers has evolved from isolated speculation at the turn of the century (Scott, 1905, 1906) to the present recognition of a wide range of transported substances and organelles (Jeffrey and Austin, 1973; Heslop, 1975). This process, or these processes, are collectively referred to as **axoplasmic** or **axonal transport**. Measured velocities are usually classified as fast or slow; **fast axonal transport** refers to velocities of about 4-20 mm/hr (100-450 mm/day) while **slow axonal transport** indicates movement at about 0.04-0.4 mm/hr (1-10 mm/day). The term axoplasmic, or axonal, "**flow**" as employed in the literature is usually applied to movement at the slow velocities. Materials are carried along the axon in both directions; movement away from the cell body is variously referred to as **orthograde**, **anterograde** or **proximo-distal** transport, and movement toward the cell body is described as **retrograde** or **disto-proximal** transport. **Somatofugal** and **somatopetal** may also be used to indicate the direction of movement and are, under some circumstances, less ambiguous terms.

Recent assessments of the literature (Bisby, 1976; Schwab and Thoenen, 1977; Kristensson, 1978, Schwartz, 1979)

indicate that retrograde (somatopetal) transport is a variety of fast axonal transport and that fast transport, in either direction, may involve particulate (Jeffrey and Austin, 1973) and, possibly, membrane bounded (Schwab and Thoenen, 1977) material. Slow transport, on the other hand, consists largely of the movement of the major structural proteins of the axoplasm: tubulin and neurofilament proteins (Lasek and Hoffman, 1976).

This review traces the development of current views on axonal transport in normal and regenerating axons with the purpose of presenting the background for the work reported in Chapters 2-5. Much pertinent research has been published since the initiation of this work. Some of this more recent literature will be discussed in the present general introduction, while certain other, more directly related, reports will be considered in the appropriate results chapters.

EARLY SPECULATIONS AND OBSERVATIONS

The dependence of the nerve fiber on the nerve cell body for its viability was first inferred by Waller in 1852 (cited by Ochs, 1975) on the basis of the degeneration he observed following transections which separated nerve processes from their somata. The first specific mention of the possibility that the axon is maintained by material conveyed along the nerve fiber seems to have been the suggestion by Goldscheider in 1894 that substances might

pass from the trophic centers of the nerves, via their axis-cylinders to their terminations.

The concept of axonal transport was expressed very explicitly in the early 1900's by Scott. On considering the common presence of a Nissl substance in nerve cells and in known secretory cells of the stomach and pancreas, and his belief that the nerve impulse was carried across the synapse by a process quite distinct from its conduction along the nerve fiber, he concluded (Scott, 1905): "The process of excitation, like that of secretion, involves, I believe, the discharge of neurosomes in the region of the synapse. Since discharge into other cells means the using up of formed material, it must be an exhaustible process, and the process of complete recovery at the synapse must depend on the integrity of the connection of the synapse with the nucleus and cell body which are the original seats of formation of the material involved in the activity." In a subsequent study of fatique in end organs of cut and uncut nerve fibers (Scott, 1906), he hypothesized "that in the body of the nerve cell a substance is formed from the nucleus and Nissl bodies which gradually passes into the nerve fibres; and also that stimulation of other cells by a nerve fibre is brought about by the passage of some of this substance into the cells on which the fibre acts." He summarized his interpretation, "And it seems to me simpler to suppose that the nerve cells secrete a substance the passage of which from the nerve endings is necessary to stimulation. The

recovery of effect after transient fatigue I attribute to the passage of a portion of this substance down the nerve fibre to the nerve ending. The absence of recovery after prolonged stimulation I attribute to the whole of the substance in the nerve fibres being used up, and to their being incapable of making more when severed from their nerve cells."

Scott's conclusions may have been drawn somewhat inferentially at the time, but as early as 1920 direct microscopic observations of moving organelles in nerve fibers were reported by Matsumoto. In cultured sympathetic neurons from embryonic chick intestine, he reported a moderate degree of movement of round and rod-shaped mitochondria stained with Janus black, and neutral red staining granules and vacuoles moving more rapidly over longer distances, sometimes in a jerky manner. These observations, however, seem to have attracted little attention, and more than 30 years elapsed before corroborating results from similar tissue culture studies began to appear (see "Somatopetal Transport").

During this period, the only comparable direct observations of intra-axonal movements, and the first to be described in neurons in vivo, appeared in Speidel's detailed light microscopic studies (1932, 1933, 1935a,b) of growing and regenerating peripheral nerve fibers in the tail fins of frog tadpoles and young salamanders. Speidel (1935a)

reported "conspicuous movements back and forth in the nerve, both of granular material and of clear neuroplasm... Such movements may be in either direction." He stated that transport of neuroplasm occurred, chiefly in the direction of the growth cones. Like the previous findings of Matsumoto, these claims too were largely ignored, and remained an isolated curiosity for many years.

Apart from these two direct experimental observations, various speculative and theoretical references to the transport of materials along axons appeared sporadically in the literature during the same period. The idea that severed portions of nerve fibers degenerated as a result of being deprived of the essential trophic influence of the cell bodies was discussed by Ramón y Cajal in 1928. Parker (1929a) suggested the possibility that neurofibrils might serve as channels for transport. He argued in favor of trophic influence by "percolation" of hormone-like substances, and estimated a velocity of about 20 mm/day, based on observations of degeneration in the lateral line nerve and organs of the catfish (Parker, 1932). Gerard (1932) considered the propagation of chemical substances or of physical changes as the two probable mechanisms by which the soma maintains the viability of the peripheral fiber. He favored the former possibility, partly because the passage of impulses was thought to accelerate rather than retard degeneration. He reasoned further that since oxygen and oxidative substrates were readily available from the nerve's

blood supply, the critical missing factor in degenerating stumps could be the "catalytic substances" necessary for their utilization. In support of this opinion, Gerard cited reports of the spread of adrenalin, bacterial toxins and viruses along the axis-cylinder, "at rates seemingly far beyond those that could be attained by simple diffusion" (see "Somatopetal Transport"). The reliability of these reports, however, was open to question since other work led to the conclusion that the route of transport was endoneurial (see Kristensson and Olsson, 1973). The possible role of the somatofugal passage of material in degenerating fibers was mentioned again by Parker and Paine (1934), by Young (1942, 1945) in connection with regenerating fibers, and had begun to attract wider interest by 1950 (see Weiss, 1950).

COMPRESSION AND CONSTRICTION EXPERIMENTS

LONG TERM EFFECTS OF PARTIAL CONSTRICTIONS

Over the six year period prior to 1948, Weiss and colleagues performed an extensive series of experiments based upon the experimental constriction of regenerating nerve fibers (Weiss and Davis, 1943; Weiss, 1944; Weiss and Taylor, 1944). A full account of the work was published in 1948 (Weiss and Hiscoe, 1948). Regenerating nerves which had undergone prolonged partial constriction inside a short elastic sleeve were observed to develop characteristic morphological responses. The diameters of the fibers were

reduced both inside the compressed zone and on the distal side of it. Proximal to the compression they developed dilatations which increased in size with their proximity to the compression. Later, similar effects were observed in constricted uncrushed nerves (Weiss, 1961). Removal of the obstruction resulted in a return of the fibers to a more normal appearance. These observations were interpreted as evidence of a proximo-distal axonal flow in normal nerve fibers, and a velocity of 1-2 mm/day was estimated from the progress of the alterations.

There appears to have been some initial uncertainty over exactly what it was that was moving. Weiss (1969b) comments in a later review: "... I myself ascribed the phenomenon to the damming of 'some essential factor produced by the nerve cell body and required for continued growth in width of the peripheral fiber.' This clearly referred to transport of something within the axon. It soon, however, became evident that this interpretation was incorrect and that ... one is dealing with a movement of the axon rather than in the axon, the axon growing forth continuously from its root in the cell body."

Weiss emphasized that the eventual outcome of the experiments with both the crushed and the uncrushed nerves was essentially identical, and that his description and conclusions consequently applied to normal intact nerve fibers as well as to mature regenerating fibers (Weiss,

1961). This position has recently been critically reassessed by Spencer (1972), who employed experimental models similar to those used by Weiss, and concluded that axonal damming and dilatation proximal to the constriction occurred only in regenerating fibers, and not in normal mature axons. These findings cast doubt on the applicability of Weiss's conclusions to normal mature fibers, and suggested that axonal damming might be a phenomenon peculiar to and perhaps caused by factors accompanying the regenerative state.

SHORT TERM ACCUMULATIONS AT COMPLETE CONSTRICTIONS

Many studies have been done on the changes which take place at constrictions in nerves. Most of these followed the publication of Weiss and Hiscoe's accumulated work (1948), but a few were done earlier (Marinesco, 1924; Sawyer and Hollinshead, 1945; Sawyer, 1946). Whereas Weiss and Hiscoe concentrated on the general morphological alterations accompanying prolonged partial constrictions, other workers focused on the detailed and more immediate internal changes, usually at complete constrictions (ligatures, crushes or cuts).

At the site of these interruptions local increases were observed in a number of enzymes, neurotransmitters and neurosecretory substances, and particulate material (reviewed by Lubińska, 1964 and Dahlström, 1971). Detection of these materials by a variety of methods (staining, biochemical assay and electron microscopy) produced

consistent evidence of accumulations on the proximal side of the interruptions, and some evidence for accumulations on the distal side (see "Somatopetal Transport").

Specified Enzymes

One of the earliest enzymes to receive general attention was acetylcholinesterase (AChE). Sawyer (1946) found an increase in enzyme activity on the proximal side from 2 to 38 days following an interruption to guinea pig sciatic nerve. Local synthesis as a possible explanation for the increased AChE was later ruled out by experiments showing that the total AChE content remained constant during the enzyme's redistribution toward the ends of a doubly ligated segment of nerve (Lubińska et al, 1964) and by the lack of effect on AChE accumulation above a ligation by local injections of the protein synthesis inhibitor cycloheximide (Frizell, Hasselgren and Sjöstrand, 1970). Increases in acid phosphatase and other esterases above interruptions were reported by Lumsden (1952); Samorajski (1957) and Gould and Holt (1961).

Oxidative and mitochondrial enzymes were also the subject of several early studies. Marinesco (1924) reported an increased activity of oxidative enzymes above lesions in dog sciatic nerve and spinal cord. Friede (1959) found accumulations of succinic dehydrogenase and TPN and DPN diaphorase in the proximal cut end of rat sciatic nerve,

accompanied by depletion of the first two from the cell bodies. Succinic, malic and lactic dehydrogenases, as well as DPN diaphorase, were found by Kreutzberg (1963) to accumulate in both proximal and distal stumps of rat sciatic nerves up to 48 hours after ligation. In adrenergic neurons, a gradual buildup of monoamine oxidase to 2.5 times its normal activity was observed proximal to a ligation, by both biochemical and histological methods (Dahlström, Jonason and Norberg, 1969). In doubly ligated hypogastric nerves of the cat, another mitochondrial enzyme, cytochrome oxidase, collected above the proximal and below the distal barriers and became redistributed toward the ends of the isolated central segment (Banks, Mangnall and Mayor, 1969). Mitochondrial distribution was studied by electron microscopy in comparable preparations, and produced results in good agreement with the biochemical enzyme assay. This general agreement extends to other electron microscopic studies of mitochondrial localization at constrictions (Dahlström, 1971). However, the early studies of mitochondrial enzyme accumulation on the distal side of constrictions produced inconsistent results.

While oxidative enzymes can be regarded as markers for mitochondria, dopamine-ß-hydroxylase is considered to be an indicator of amine storage granules. This enzyme too has been reported to accumulate on the proximal side of ligations in adrenergic neurons (Laduron and Belpaire, 1968; Livett, Geffen and Rush, 1969; Geffen, Livett and Rush,

1969).

Several enzymes which were thought to be soluble in the cytoplasm were also detected at constrictions in elevated amounts. DOPA decarboxylase exbihited a delayed accumulation 2 days (Dahlström and Jonason, 1968) and 1 day (Laduron, 1970) after ligation in rat sciatic nerves. Skangiel-Kramska, Niemierko and Lubińska, (1969) observed a limited increase in phosphoglucoisomerase activity. Choline acetylase (ChAc) was found to increase substantially proximal to sections of sheep and cat cervical sympathetic nerve (Hebb and Waites, 1956) and the sciatic nerve of the goat (Hebb and Silver, 1961, 1963). Similar results were obtained by Frizell, Hasselgren and Sjöstrand (1970) in hypoglossal and vagus nerves of the rabbit. Opinion was divided over whether to interpret these elevated enzyme levels as a consequence of interrupted axonal transport or as some kind of synthetic or other localized reaction to the experimental procedure.

Neurotransmitters and Neurosecretory Substances

During the 1950's and 1960's evidence was obtained that a variety of neurotransmitters and neurosecretory materials may collect at axonal interruptions. Histochemically and biochemically detected proximal accumulations of neurotransmitter substances were reported for acetylcholine in cat ventral roots (Diamond and Evans, 1960) and rabbit

sciatic nerves (Evans and Saunders, 1967), for substance P in rabbit auricular and sciatic nerves (Holton, 1960), for 5-hydroxytryptamine and dopamine in the spinal cords of rats and other mammals (Dahlström and Fuxe, 1964b; 1965), for L-dihydroxyphenylalanine and dopamine (Sotelo and Taxi, 1971) and for noradrenaline in a number of different species (Dahlström and Fuxe, 1964b; Kapeller and Mayor, 1966a,b; Dahlström, 1965; Dahlström and Häggendal, 1966, 1967; Banks, Mangnall and Mayor, 1969). Similarly, accumulations of neurosecretory materials were detected above cut and blocked sites in the hypothalamic-hypophysial tract in Anura (Hild, 1951), in the dog (Scharrer and Wittenstein, 1952; Hild and Zettler, 1953) and in the rabbit (Christ, 1962), and in the analogous neurosecretory neurons of **Leucophaea** (Scharrer, 1952) and **Calliphora** (Thomsen, 1954). Again, these findings were variously interpreted as indications of transport, or of local reactions to the injury.

Particulate Material

The same dichotomy of interpretations applied to light and electron microscopic observations of increases in the numbers of axonal organelles and particles at nerve constrictions. These included mitochondria, lysosomal organelles, vesicles, vacuoles, membranous elements and granular material (Lubińska, 1964; Kreutzberg, 1967). It is difficult to discuss these components individually, because in addition to the problems of precisely defining the

structures in normal axons, the experiments concerned damaged axons where particulate material is highly condensed and partial degeneration may have begun. The elevated concentrations of particulate matter were regarded as local products by Inoue (1960), Schlote and Hager (1960) Wechsler and Hager (1962) and Wettstein and Sotelo (1963), and as manifestations of interrupted movement by Van Breemen, Anderson and Reger (1958), Weiss, Taylor and Pilla (1962), Lubińska et al (1963) and Melamed and Trujillo-Cenóz (1963), while De Robertis and Sotelo (1952), Estable, Acosta-Ferriera and Sotelo (1957) and Hay (1960) were not as committal.

Interpretation of Accumulations at Constrictions

Interpretation of the accumulations of enzymes, neurosecretory substances and particles to blockage of a normal axoplasmic migration was hampered by the fact that axonal transport, especially bidirectional rapid transport, was at the time not established as an essential and integral neuronal process. The reluctance to recognize the potential contribution of axonal transport was of course most pronounced in connection with accumulations found on the distal sides of interruptions, since the methods in use at the time were strongly biased toward the detection of proximo-distal transport. Another factor was the extensive but rather restrictive influence of Weiss and Hiscoe. Their influential model, with its exclusively slow velocity and

explicitly proximo-distal direction (Weiss, 1944; Weiss and Hiscoe, 1948; Weiss, 1970) was for some time the accepted concept of axoplasmic transport. In keeping with this model, material appearing too rapidly on the proximal side, or appearing at all on the distal side, had to be explained as some form of local reaction (Christ, 1962; Wechsler and Hager, 1962; Pellegrino de Iraldi and de Robertis, 1970). At times the attitude even seemed to prevail that the side on which the accumulation appeared could be used as a criterion of whether it had arrived by axonal transport or had been produced by a local reaction to the constriction (Lee, 1963; Kapeller and Mayor, 1969a,b).

Those who considered the accumulations to be partially or wholly accounted for by processes other than interrupted transport have suggested almost every conceivable alternative. The most frequent of these was the transformation or remodeling of local pre-existing structures (Hild, 1951; Inoue, 1960; Schlote and Hager, 1960; Webster, 1962; Lee, 1963; Wettstein and Sotelo, 1963; Blümcke and Niedorf, 1964; 1965b; Holtzman and Novikoff, 1965; Blümcke, Niedorf and Rode, 1966; Schlote, 1966; Kreutzberg, 1967; Pellegrino de Iraldi and de Robertis, 1968; 1970; Kapeller and Mayor, 1969a,b; Geffen and Ostberg, 1969; Pellegrino de Iraldi and Rodríguez de Lores Arnaiz, 1970; Morris, Hudson and Weddell, 1972; Matthews, 1973; Sotelo and Taxi, 1973; Duce and Keen, 1976). In addition, all the following possibilities have been mentioned or

advocated: "activation" of axoplasmic components (Wechsler and Hager, 1962; Holtzman and Novikoff, 1965; Skangiel-Kramska, Niemierko and Lubińska, 1969); local synthesis (Christ, 1962; Evans and Saunders, 1967; Kreutzberg, 1967; Pellegrino de Iraldi and de Robertis, 1968; 1970; Skangiel-Kramska, Niemierko and Lubińska, 1969; McLean and Burnstock, 1972); self-propagation of organelles or structures by growth or division (Webster, 1962; Lee, 1963; Melamed and Trujillo-Cenóz, 1963; Wettstein and Sotelo, 1963; Blümcke and Niedorf, 1965b; Blümcke, Niedorf and Rode, 1966; Kapeller and Mayor, 1969a); swelling (Lee, 1963; Blümcke and Niedorf, 1964); metabolic changes (Inoue, 1960; Blümcke, Niedorf and Rode, 1966); local acquisition, involving uptake by axolemmal endocytosis and transfer of material from Schwann cells or myelin (Inoue, 1960; Webster, 1962; Melamed and Trujillo-Cenóz, 1963; Wettstein and Sotelo, 1963; Holtzman and Novikoff, 1965; Morris, Hudson and Weddell, 1972; Sotelo and Taxi, 1973); aggregation or coalescence of intra-axonal material (Lee, 1963; Schlote, 1964; Blümcke and Niedorf, 1965a; Blümcke, Niedorf and Rode, 1966); mechanical force or pressure gradient (Kapeller and Mayor, 1969a,b); electrical gradient (electrophoresis or "galvanotaxis") (Friede, 1964a,b; Weiss, 1970); unspecified reactions to constriction (Estable, Acosta-Ferreira and Sotelo, 1957; Schlote, 1964; Lever et al, 1970); and experimental inadequacies (McLean and Burnstock, 1972).

Interruption of transport gradually became a more attractive explanation with the growing documentation of fast transport velocities and bidirectional transport of material through the axoplasm. In recent years, axonal transport has been considered to partly or wholly account for accumulations of various organelles (Martinez and Friede, 1970; Matthews, 1973), amine storage granules and noradrenaline (Dahlström and Fuxe, 1964a; Dahlström, 1965; 1967b; Dahlström and Häggendal, 1966; Banks, Mangnall and Mayor, 1969; Brimijoin, 1977), acetylcholine (Dahlström et al, 1974), specific enzymes including acetylcholinesterase (Zelená and Lubińska, 1962; Lubińska et al, 1963; Lubińska and Niemierko, 1971), dopamine-ß-hydroxylase (Brimijoin, 1975; Brimijoin and Wiermaa, 1977b, and tyrosine hydroxylase (Brimijoin and Wiermaa, 1977b) and ^{14}C leucine labelled proteins (Bray, Kon and Breckenridge, 1971) on the proximal side of axonal obstructions, and of ^{14}C leucine labelled proteins (Bray, Kon and Breckenridge, 1971), ^{3}H leucine labelled proteins (Abe, Haga and Kurokawa, 1974), amine storage granules (Dahlström, 1965), dopamine-ß-hydroxylase (Brimijoin and Helland, 1976), acetylcholinesterase (Zelená and Lubińska, 1962; Lubińska et al, 1963; Lubińska and Niemierko, 1971) and possibly acetylcholine (Dahlström et al, 1974) on the distal side. In segments of axons isolated by two separate interruptions, bidirectional axonal transport has been proposed as an explanation for the redistribution from the center of the segment to both the

ends, of mitochondria (Zelená, 1968; 1969; Banks, Mangnall and Mayor, 1969; Friede and Ho, 1977), mitochondrial hexokinase and glutamic dehydrogenase (Partlow et al, 1972) and cytochrome oxidase and adenosine triphosphate (Banks, Magnall and Mayor, 1969) acetylcholinesterase (Zelená and Lubińska, 1962; Lubińska et al, 1963; Lubińska et al, 1964; Partlow et al, 1972) and ^{14}C leucine labelled proteins (Bray, Kon and Breckenridge, 1971).

TRANSPORT OF RADIOACTIVELY LABELLED TRACERS

Attempts to introduce radioactive isotopes into neurons and to follow their subsequent movements were begun between 1945 and 1950 (see Gerard, 1950; Weiss, 1969a). The first reports appeared in 1951 (Samuels et al, 1951; Shepherd, 1951, cited by Weiss, 1969a) and the next, several years later (Waelsch, 1958; Koenig, 1958; Ochs and Burger, 1958). The technique has grown in recent years to be the most widely used approach in the study of axonal transport, and has produced a wealth of evidence for the transport of a wide variety of neuronal materials. Despite its convenience and reliability, however, it remains subject to certain fairly strict limitations (discussed later), and as a result a perhaps disproportionate emphasis has been placed upon proteins and glycoproteins transported in the somatofugal direction.

SLOW TRANSPORT

The first radioisotope to be employed as an indicator of axoplasmic transport was ^{32}P. Following intraperitoneal injection of radioactive orthophosphate into guinea pigs, Samuels et al (1951) found a gradient of radioactivity established along the sciatic nerve, suggesting that some of the phosphorus had become incorporated into neuronal compounds which moved along the nerve in a proximo-distal direction. Several years later, Waelsch (1958) produced evidence of a progressive proximo-distal shift of protein by systemic injection of ^{14}C labelled amino acids as precursors.

In the same year, successful local injections of radioactive tracers into specific regions containing the cell bodies of the neurons under study were reported. Koenig (1958) injected labelled methionine or glycine intra-cisternally, while Ochs and Burger (1958) injected ^{32}P orthophosphate directly into the ventral horn, and in both cases a proximo-distal gradient of activity was found in the appropriate nerves. Such localized introduction of precursors permitted improved definition without the problem of high background levels of radiation accompanying systemic injections. A number of subsequent studies continued to provide comparable evidence for the incorporation of radioactive amino acids into proteins which appeared to then move somatofugally along the nerves at a slow velocity (Droz and Verne, 1960; Miani, 1960; Verne and Droz, 1960; Lajtha,

1961; Weiss, 1961; Ochs, Dalrymple and Richards, 1962; Rahmann, 1965; Taylor and Weiss, 1965; Austin, Bray and Young, 1966; Ochs, Johnson and Ng, 1967; Weiss, 1967a; Weiss and Holland, 1967; Rahmann, 1968).

An important further improvement in technique was achieved with the introduction of tritiated amino acid as the precursor. Using locally injected ^{3}H leucine and autoradiography, Droz and Leblond (1962, 1963) definitively demonstrated somatic incorporation of amino acids into protein and subsequent transport down the axon with a velocity in the vicinity of 1 mm/day. This technique allowed them to confirm that the transported protein was located inside the axons.

TRANSPORT AT HIGHER VELOCITIES

The first measurements of radioactively labelled compounds moving along nerve fibers at velocities higher than a few millimeters per day were reported by Miani (1962, 1963, 1964). Using as a marker ^{32}P, applied locally to the calamus scriptorius of the fourth ventricle he obtained velocities of 39-72 mm/day for the distal movement of specific phospholipids in rabbit hypoglossal and vagus nerves, which he considered too fast to identify with axon growth and therefore interpreted as movement not of, but through, the axon. This discovery was followed by a report of ^{3}H leucine labelled protein moving at two distinctly different rates, approximately 1-2 and 100-200 mm/day, in

rat ventral root and cat dorsal root fibers (Lasek, 1966). Later an intermediate velocity was suggested in rat motoneurons (Lasek, 1968b), and a velocity exceeding 500 mm/day was reported for cat dorsal root ganglion cells (Lasek, 1968a). Grafstein (1967) found autoradiographic evidence of two rates of protein movement in goldfish optic nerve fibers, about 0.4 and 10 mm/day, which differed by a factor of at least 25. This "fast" rate too, was later revised upward (McEwen and Grafstein, 1968) to 40 mm/day or more. Kerkut, Shapira and Walker (1967) studied the transport of labelled glutamate in an isolated CNS-nerve-muscle preparation from snails and frogs, finding transport rates of 720 and 120 mm/day, respectively. Velocities above 1 mm/day were reported in a number of other studies (Goldberg and Kotani, 1967; Barondes, 1968; Karlsson and Sjöstrand, 1968; Livett, Geffen and Austin, 1968; Young and Droz, 1968; Bray and Austin, 1969; Ochs and Johnson, 1969; Ochs, Sabri and Johnson, 1969). It thus became abundantly clear that labelled compounds were transported out of the soma at relatively high velocities, but with the various different markers, species, and nerves under study, there was a bewildering variety of reported velocities. This diversity of observed rates is still characteristic of research on axonal transport, although some velocity ranges have come to be regarded as fairly standard (see "Introduction").

CHARACTERISTICS OF THE TECHNIQUE

The use of radioactive tracers has gone on to become the most widely utilized method in the study of axonal transport, and has provided a great deal of useful information (recent reviews by Lasek (1970b), Ochs (1972a), Jeffrey and Austin (1973) and Heslop (1975)). It is a reliable technique which allows transport of proteins and other molecules to be monitored in intact, normally functioning neurons. However it has its disadvantages, and these tend to limit the kinds of information which it can provide.

Experiments utilizing radioactive tracers usually depend upon biosynthetic reactions to incorporate the labelled precursors into the transported substances and structures, and therefore are largely confined to the study of somatofugal transport. Accuracy and precision are compromised by the time lags involved in some combination of uptake through the cell membrane, incorporation into transported substances or organelles, and possible storage before transport. In addition, extensive averaging effects are involved, since analyses are usually performed on segments of whole nerves, which include sensory and motor, and myelinated and unmyelinated axons of various sizes and neurotransmitter types. Furthermore, the precursor may have been taken up by a variety of different molecules or be associated with different organelles, and may have left the cell bodies asynchronously. A final difficulty is imposed by

the poor spatial and temporal resolution afforded by analysis based upon segments of nerve several millimeters in length and obtained at discrete time intervals.

SOMATOPETAL TRANSPORT

While the notion of material moving from its site of synthesis in the nerve cell bodies to its site(s) of utilization in the axons may have been a fairly "natural" idea, there were early indications that movement of certain materials in the opposite direction was also a possibility.

CENTRIPETAL SPREAD OF NEUROTROPIC VIRUSES AND BACTERIAL TOXINS

There was considerable interest and controversy around the early 1900's concerning the route of entry and transmission of several neurotropic viruses and bacterial toxins, and for several of these agents it was popular to propose a direct disto-proximal propogation along the peripheral nerves. Opinion was strongly divided, however, on whether transmission took place next to the neurons in the neural lymphatics, endoneural or perineural spaces or whether the materials entered the axons and ascended inside them. Since there was no established knowledge of axonal transport at that time, it was difficult to argue in favor of the latter possibility; nevertheless, this interpretation was advanced by Goodpasture and Teague (1923), Goodpasture (1925b), Sabin (1937) and Sabin and Olitsky (1937) for the

spread of Herpes simplex, febrilis, B, pseudorabies, vesicular stomatitis and eastern equine encephalomyelitis viruses, by Fairbrother and Hurst (1930) and Bodian and Howe (1941) for poliomyelitis virus, by Goodpasture (1925a) for rabies virus, and by Meyer and Ransom (1904), Thiele and Embleton (1914), and Teale and Embleton (1919) for tetanus toxin. Marie and Morax, and Golla were also cited in this connection by Yuien (1928). The research on tetanus was reviewed by Zacks and Sheff (1970).

The validity of some of these studies has been challenged by subsequent critics, but some of the original conclusions have endured to the present time and have received further support from recent studies of Herpes simplex virus (Kristensson, 1970a; Kristensson, Lycke and Sjöstrand, 1971; Cook and Stevens, 1973; Kristensson, Ghetti and Wisniewski, 1974) and of tetanus toxin (see reviews by Schwab and Thoenen, 1977; Kristensson, 1978), while other conclusions regarding such agents as rabies and polio viruses have yet to be disproven. It is certain the early workers faced intellectual problems in proposing an intra-axonal ascent of pathogenic agents at a time when there was no knowledge of the axonal transport process. These problems were not diminished by the ensuing disproportionate emphasis and misconception of axonal transport as an essentially proximo-distal movement, since the agents would then have to move "against the current" (Baer, Shanthaveerappa and Bourne, 1965; Kristensson, Lycke

and Sjöstrand, 1971).

EVIDENCE FROM CONSTRICTION STUDIES

A number of the early constriction experiments supported the idea of somatopetal axonal transport. Although accumulations of material on the distal side of an axonal constriction were generally smaller, and were observed with less regularity than those on the proximal side, analyses under appropriate conditions revealed distal increases of acid phosphatase and non-specific esterase (Gould and Holt, 1961), DPN diaphorase, lactic, malic and succinic dehydrogenase (Kreutzberg, 1963), acetylcholinesterase (Zelená and Lubińska, 1962; Lubińska et al, 1963; Kasa, 1968; Lubińska and Niemierko, 1971), monoamine oxidase (McLean and Burnstock, 1972), catecholamines (Dahlström, 1965, 1967a), radioactively labelled compounds (Lasek, 1967), neurosecretory substances (Hild, 1951; Christ, 1962; Diepen, 1962) and particulate material (Lubińska et al, 1963; Schlote, 1966; Zelená, Lubińska and Gutmann, 1968; Kapeller and Mayor, 1969a). A number of other studies, employing double constrictions spaced a short distance apart, showed accumulations at both ends of the isolated segment, with reductions in the central portion, of noradrenaline (Dahlström, 1965, 1967a), acetylcholinesterase (Lubińska et al, 1964; Skangiel-Kramska, Niemierko and Lubińska, 1969), cytochrome oxidase and adenosine triphosphate (Banks, Mangnall and Mayor, 1969),

radioactively labelled compounds (Miani, 1964; Lasek, 1967) and mitochondria (Blümcke, Niedorf and Rode, 1966; Zelená, 1968; Banks, Mangnall and Mayor, 1969). This sort of evidence was consistent with the idea of somatopetal movement, but it was not conclusive. It was questionable whether the observed materials had in fact been moving along the nerve before the constriction was applied, or whether the material represented an abnormal accumulation of products induced by the injury. Several versions of the latter possibility were advanced: degenerative changes (Hild, 1951), interconversion and morphological alterations of subcellular organelles and structures (Schlote, 1966), and injury-generated electrophoretic forces (Friede, 1964a,b; Weiss, 1970). Local, injury-induced, synthesis was proposed by Christ (1962), and a specific example, the induction of mitochondrial monoamine oxidase synthesis in response to release of noradrenaline in the damaged region, was discussed by McLean and Burnstock (1972). This idea, however, contrasted with the measurements made by Lubińska et al (1964) which had shown that the total amount of enzyme (AChE in this case) in an isolated nerve segment remained constant during its redistribution to the two ends. Bray, Kon and Breckenridge (1971) obtained evidence that material arriving at nerve terminals by somatofugal transport might reverse direction and that a comparable situation might develop in the artificial terminals created by axonal occlusions. This possibility could help to explain events

distal to the proximal member of a double constriction, but in the absence of a more distal constriction, the experiment provides support not for an abnormal event, but for somatopetal movement as a normal process.

MICROSCOPIC OBSERVATION OF ORGANELLE MOVEMENTS IN AXONS

Direct evidence of sustained somatopetal movement in nerve axons appeared in 1953, in the form of Hughes' filmed light microscopic observations of tissue-cultured spinal ganglion cells from the chick. Matsumoto (1920) had previously noted that intra-axonal movements of mitochondria were limited, and while granules were said to exhibit considerable longitudinal movement, the direction was not specified. Lewis had reported pinocytosis in the amoeboid tips of cultured axons (1945) and referred to the possibility of axonal organelle movement (1950), but Hughes provided the first definite account, describing the movement of pinocytotic vacuoles, away from the neurite tip and towards the cell body, as continuous over distances of at least 12-28 µm. The distances may have been greater, since the 0.3-0.7 µm vacuoles were usually lost from focus before any indication of impaired progress appeared. Their velocities ranged from 0.06 to 0.28 µm/sec.

Pinocytosis at the growth cone and somatopetal movement of vacuoles were also documented by Nakai (1956), using time lapse cinemicrography of cultured spinal ganglion cells from chick embryos. The progress of the vacuoles was described as

discontinuous, with a velocity of about 0.02 μm/sec. In addition, mitochondria and other granules were observed to shift in both directions between the perikaryon and the axon. Similar observations were made by Godina (1956; cited by Dahlström, 1965). Detailed filmed records of comparable movements were produced by Hayden, Pomerat and Smith (1954) and Pomerat et al (1967), and observations of a similar nature were reported by several other authors (cited by Lubińska, 1964; Pomerat et al, 1967).

Bidirectional particle movements at velocities much higher than those previously reported were recorded on motion picture film by Burdwood (1965). In neurons of cultured dorsal root ganglia, particles generally moved at rates from 1 to 5 μm/sec, with some rates in excess of 20 μm/sec (Rebhun, 1972). Since no details concerning the method of measurement were given, it may be that the particle movements were irregular or saltatory and that the high values are not average velocities but maximum instantaneous velocities. Even so, the higher values are exceptional, and have not been reported since for optically detectable particles. Tissue-cultured fibers from embryos of three different amphibian species were studied by Berlinrood, McGee-Russell and Allen (1972). Interspecific differences were found to exist, but the highest range of instantaneous velocities found was 0.6 to 2.25 μm/sec. The 1.0-1.5 μm diameter particles moved bidirectionally with pathlengths from 4 to 63 μm which had no correlation with

velocity. Leestma (1976) recorded movements of spherical particles 0.3-0.8 µm in diameter somatopetally at a mean velocity of 1.03 µm/sec and somatofugally at 1.07 µm/sec in cultured embryonic mouse neurites. Observations of a similar nature were made in a variety of primary neuronal cultures and continuous cell lines by Breuer et al (1975).

In spite of the varied and accumulating evidence in support of somatopetal transport, it was still possible to deny its existence in normal mature nerves of adult animals. It could be argued that the viruses and toxins may have been moving in the endoneurial or perineurial spaces (see Kristensson and Olsson, 1973; Yuien, 1928; Zacks and Sheff, 1970), that the studies on interrupted fibers, having introduced major side effects unrelated to axonal flow; were crude and led to confusion (Weiss, 1972), and that there was no justification for extending conclusions from tissue cultured neurons, which were immature and actively growing in an artificial and abnormal environment, to neurons in the substantially different mature state (Weiss, 1967b). Three further methodologies however have finally overcome any lingering doubts; these will be briefly discussed below.

One of the most recent developments in the study of axonal transport has been the achievement of direct microscopic observation of particle movements along functional axon segments isolated from mature peripheral nerves, immediately following their removal from normal

animals (Smith, 1971, 1972a). These and several subsequent reports are discussed in detail in Chapter 2. However one of the most interesting and dramatic results to emerge from this approach was that most of the particles detected moved somatopetally at velocities of about 1 μm/sec.

RADIOACTIVE LABELLING OF SOMATOPETALLY MOVING MOLECULES

Although the introduction of radioactive amino acids at the axon terminals is severely limited compared to somatic labelling, it has been accomplished in certain limited forms. The dependence of the technique upon the incorporation of radioactive precursors of biological macromolecules has recently been circumvented by the use of pre-labelled macromolecules. Somatopetal transport of radioactive compounds has also been studied following the suspected reversal of somatofugally moving material at the nerve terminals or at artificial barriers. This latter approach is discussed in Chapter 4.

In the experiments of Kerkut, Shapira and Walker (1967), contiguous organ systems consisting of brain or spinal cord, nerve trunk, and muscle were removed intact from both snails and frogs, and transfered into a chamber designed to isolate the muscle from the CNS, except for the nerve running between them. In both preparations, when ^{14}C labelled glutamate was added to the muscle bathing medium, radioactive material was found in the CNS tissue within 24 hours. Precautions were taken to prevent leakage or

diffusion by possible extra-axonal routes, and autoradiography of the nerve trunk showed labelled material located mainly in the axons of the nerves.

A similar centripetal migration of radioactivity was demonstrated under entirely in vivo conditions by Watson (1968b), along axons of the hypoglossal nerve of the rat. From 6 to 12 hours after injection of ^{3}H lysine into the geniohyoid muscle, radioactivity appeared in the nerve branch innervating the muscle, and after 48 hours, a small proportion of the axons in the more proximal hypoglossal nerve was heavily labelled. It was not known whether the lysine gained access and underwent transport as the free amino acid, or whether its transport depended upon synthetic reactions. The possibility of limited ribosomal synthesis of protein or incorporation into existing macromolecules in the axon terminals has been discussed by Lubińska (1964), Barondes (1969, 1974) and Heslop (1975). Nonribosomal incorporation of amino acids into neuronal protein has been reported by Carlin (1977).

The attempts to introduce tracer amino acids at the periphery were followed by the use of previously labelled radioactive proteins. After injection of ^{125}I labelled nerve growth factor (NGF) in the region of adrenergic nerve terminals in the iris of mice, there was a preferential accumulation of radioactivity in the cell bodies of the ipsilateral superior cervical ganglion, peaking at about 16

hours (Hendry et al, 1974). This accumulation could be prevented by transection of the nerve fibers, intraocular injection of colchicine, and perhaps (see Fillenz et al, 1976) by 6-hydroxydopamine treatment of the adrenergic terminals. Accumulation also occurred in sympathetic neuron cell bodies following injection into the submaxillary gland (Stöckel, Paravicini and Thoenen, 1974), and was shown by autoradiography to be transported in sympathetic axons (Iversen, Stöckel and Thoenen, 1975). A similar terminal uptake and proximal transport of proteins was indicated in studies using ^{125}I tetanus toxin (Price et al, 1975; Stöckel, Schwab and Thoenen, 1975; Wellhöner, Erdmann and Wiegand, 1976; Neale and Dimpfel, 1976; Green, Erdmann and Wellhöner, 1977) and radioactively labelled dopamine-β - hydroxylase antibodies (Fillenz et al, 1976). The physiological significance of these and related reports has been discussed by Schwab and Thoenen (1977).

In addition to retrograde transport of radioactive proteins and amino acids, evidence has been reported for the somatopetal migration of injected labelled nucleotides or derivatives (Wise and Jones, 1976; Hunt and Kunzle, 1976).

SOMATOPETAL TRANSPORT OF NON-RADIOACTIVE TRACERS

Success has been achieved recently in attempts to demonstrate neuronal uptake and somatopetal transport of non-radioactive tracer substances applied in vivo to the region of the nerve terminals (reviewed by Kristensson and

Olsson, 1973; Kristensson, 1978). Kristensson and co-workers (Kristensson, 1970b; Kristensson and Olsson, 1971b,c,d; Kristensson, Olsson and Sjöstrand, 1971) found that intramuscular injections of albumin coupled with the fluorescent dye Evans blue (EBA) were taken up by motor neurons and transported towards the cell bodies. It was thought that the uptake probably occurred by pinocytosis at the nerve terminals (Kristensson and Olsson, 1971a,d; Kristensson, Olsson and Sjöstrand, 1971), and the transport rate was estimated to be 120 mm/day, in the rabbit hypoglossal nerve. This somatopetal velocity was only about half that of about 300 mm/day reported by Sjöstrand (1970) for labelled proteins moving in the distal direction in the same nerve, and transport in both directions could be blocked by anoxia, colchicine and crushing.

Shortly after the tracer properties of EBA were discovered, a second histologically detectable protein was found to behave in much the same manner. Horseradish peroxidase (HRP), which had been observed to enter nerves at neuromuscular junctions (Zacks and Saito, 1969) was found to subsequently appear in nerve fibers and cell bodies in the form of granules, vesicles, vacuoles and multivesicular bodies (Kristensson and Olsson, 1971a,b,d; Kristensson, Olsson and Sjöstrand, 1971). These experiments were performed using the tongue and gastrocnemius muscle of mammals (rabbits, rats and mice), but the same phenomenon was observed in frogs (Ceccarelli, Hurlbut and Mauro, 1972;

Litchy, 1973) and chicks (LaVail and LaVail, 1972, 1974). In the latter experiments, HRP was traced along two CNS pathways, from terminals in the retina to cell bodies in the isthmo-optic nucleus, and from terminals in the optic tectum to retinal ganglion cell bodies. Electron microscopy showed the HRP in large membrane bounded pinocytotic vesicles, multivesicular bodies, cup-shaped organelles and tubules of smooth endoplasmic reticulum. Transport occurred at a velocity in the vicinity of 72-84 mm/day, and was inhibited by colchicine.

HRP labelling has become the most widely used technique for tracing retrograde axonal transport, due to its relative generality and reliability; so much so, in fact, that it has been adopted by neuroanatomists as a valuable tool for investigating neuroanatomical pathways (Cowan and Cuenod, 1975; Kim and Strick, 1976).

SELECTIVITY OF SOMATOPETAL TRANSPORT

Tracers taken up at axon terminals have done much to elucidate the subject of retrograde axonal transport, yet there are many cases where the outcome has been negative. Selectivity of uptake is no doubt an important limiting factor in these attempts. Specificity is displayed by nerve endings both with respect to the condition or types of neurons, and to the substances applied. In the former case, Kristensson (1970b) found that the uptake and somatopetal transport of intramuscularly injected EBA, readily

demonstrable in suckling mice, was confined to the first few weeks of life, and suggested a possible connection between this difference and the age dependence of murine susceptibility to peripherally inoculated Herpes simplex virus. The exclusion of EBA from the neurons of adult mice could have been mediated by a diminishing pinocytotic capacity at the maturing axonal neuromuscular terminals, or by the decreasing permeability of the perineurium to protein tracers (Kristensson and Olsson, 1971c). Hanson, Tonge and Edström (1975) were unable to demonstrate uptake and transport of HRP in an in vitro sciatic nerve-gastrocnemius muscle preparation from the frog, although the tracer was carried to cell bodies of the hypoglossal nucleus in vivo within 24 hours of injection into the tongue. In another experiment (Fillenz et al, 1976), adrenergic sympathetic neurons were shown to take up and transport injected DBH antibodies from the anterior eye chamber of rodents, while sensory and motor neurons did not respond to antibody injections into the forepaw and deltoid muscle.

On the other hand, specificity with respect to the applied substance was evident in the work of Stöckel, Paravicini and Thoenen (1974) and Stoeckel, Schwab and Thoenen (1975), where nerve growth factor, but none of the other proteins insulin, ferritin, cytochrome c, HRP, bovine albumin or ovalbumin, were carried from the anterior eye chamber to cell bodies of the superior cervical ganglion, and where the transport of NGF itself was inhibited by

relatively small chemical changes in the molecule. Factors thought to be involved in the selective nature of the uptake are discussed in detail by both Schwab and Thoenen (1977) and Kristensson (1978).

COMMENT

The development of the present understanding of axonal transport has been outlined. The course of the research has been: (1) to obtain evidence for an active transport process, (2) to define velocities and directions, (3) to identify the transported materials, (4) to investigate mechanisms, and (5) to determine the functions of the process. The first three aspects have been reviewed in some detail. Emphasis has been placed on the technical approaches to the problem since advances have obviously been associated with the introduction of new techniques. A second reason for this emphasis is that one of the newer techniques (optical) has been used exclusively in the experimental work in this thesis, and its use might be expected to generate a somewhat different view of axonal transport than existed earlier.

Mechanisms have not been pursued since a) they are not of great significance to the thesis b) they are not well understood. There is almost no evidence which allows a mechanism for slow axonal transport to be proposed. Fast transport, both somatofugal and somatopetal, is sensitive to mitotic inhibitors and thus is thought to depend on the integrity of axonal microtubules (Hammond and Smith, 1977).

A role for endoplasmic reticulum in fast axonal transport has also been proposed (Droz, Rambourg and Koenig, 1975).

The functions of axonal transport are of consequence to this thesis, particularly those aspects of the transport system which may play a role in axonal degeneration and regeneration. This specific aspect of the function of axonal transport is reviewed below.

AXONAL TRANSPORT DURING DEGENERATION AND REGENERATION

TRANSPORT IN DISTAL PORTIONS OF DAMAGED AXONS

It has been shown that in the distal portions of neurons separated by sectioning or by ligation from their perikarya, the more obvious characteristics of axonal transport are not immediately affected. Based upon a review of microscopic observations of growing neurons both in vivo and in vitro, Lubińska (1964) stated that "Before it finally degenerates, a cut nerve fibre continues for many hours the activity it manifested before the transection." This activity included back and forth movements of granules along the axon, and ascent of droplets taken in at the tip of the axon by pinocytosis. As Lubińska pointed out, this behavior was consistent with the continuation of streaming activity observed in the anucleate cytoplasm of various other types of plant and animal cells. It was consistent as well with the idea that materials accumulating on the distal side of nerve interruptions were carried there by the transport system (see "Somatopetal Transport"). It also complemented

findings that the time course of several changes at the synapse depended upon the length of the peripheral stump left connected to the end organ (Lubińska, 1964). The persistence of organized movement in various forms of isolated cytoplasm was interpreted by Lubińska and others to indicate that the factors generating the movement were present generally throughout the cytoplasm itself.

A comparable conclusion was arrived at through studies based on the transport of radioactively labelled materials along nerve fibers. Ochs and Ranish (1969) concluded that the mechanism of fast transport was locally and uniformly present all along the length of the axons, and that continued transport depended upon oxidative metabolism locally in every part of the axon (Ochs, 1971b). Ochs postulated a hypothetical model for the mechanism of axonal transport (Ochs, 1971a, 1972a), all the elements of which are normal constituents or inclusions of axoplasm.

Based upon reports of continued fast transport in isolated nerve segments, Frizell and Sjöstrand (1974b) and Frizell, McLean and Sjöstrand (1975) also concluded that the process was not interfered with initially by section or ligation. However, immediate cessation of the progress of slowly migrating proteins upon isolation of vagus nerve fibers from their cell bodies by ligation or colchicine treatment (Frizell, McLean and Sjöstrand, 1975) suggested that slow transport may differ from rapid transport in

requiring maintained contact with the cell bodies.

SIGNIFICANCE OF AXONAL TRANSPORT IN REGENERATING NEURONS

While it was reasonable to assume that axonal transport would eventually break down in the degenerating distal portions of axons separated from their cell bodies, actual observations of the process under these conditions were almost non-existent. Likewise, little information was available concerning transport in the regenerating proximal part of the axon, but it stood to reason that the system specialized for moving proteins and other materials along the normal axon should be a factor of even greater importance in supplying materials during a time of increased synthetic activity.

Several other considerations were relevant to the possible readjustment of axonal transport in response to the altered conditions of regeneration. While the demand for molecules to be incorporated into the growing structure could be expected to increase, there were also logical and experimental grounds to predict a decrease during regeneration in the demand for proteins involved in synaptic transmission (Schwarzacher, 1958; Navaratnam, Lewis and Shute, 1968; Boyle and Gillespie, 1970; Matthews and Raisman, 1972; Cheah and Geffen, 1973; Karlström and Dahlström, 1973; Frizell and Sjöstrand, 1974b). Hence it was not necessarily a simple increase in quantity of transported material to be anticipated, but perhaps a qualitative shift

from substances involved in the transmitter function of the neuron to those involved as structural elements of the cell.

While both increased synthesis of structural proteins and decreased transmitter synthesis related primarily to somatofugal transport, there were also considerations suggesting the possible involvement of somatopetal transport in regeneration. It had been shown by several investigators that pinocytosis of fluid from the surrounding medium into vacuoles, and subsequent proximal movement along the axon, were characteristic features of growing neurons in cultures of embryonic nervous tissue (Matsumoto, 1920; Lewis, 1945; Hughes, 1953; Nakai, 1956; Pomerat et al, 1967; and others). A few years later indications began to appear that injured and regenerating axons of mature neurons in vivo could take up exogenous proteins applied at their tips and transport them back to the cell bodies (Kristensson and Sjöstrand, 1972; Kristensson and Olsson, 1974; De Vito, Clausing and Smith, 1974). These observations suggested that the components of somatopetal transport might be altered during regeneration by the addition of extra-axonal material "sampled" at the growth cone by pinocytosis or other uptake mechanisms.

The possibility of communication between the axon and the cell body, mediated by somatopetal axonal transport, was relevant to two unexplained properties of damaged neurons, viz., the initiation of the chromatolytic response to axonal

injury, and the possible regulation of the regenerative process by the conditions at the axon tip. Cragg (1970) reviewed possible hypotheses for the initiation of chromatolysis, among which the possibility of a role played by retrograde axonal transport figured prominently. Other authors were also of the opinion that a change in the substances reaching the cell bodies by retrograde transport deserved serious or foremost consideration as the signal for chromatolysis (Ochs, 1972b; Kristensson and Olsson, 1973). The idea had also been expressed that, once underway, the regenerative response was likely to be subject to some form of control by the exchange of chemical information between the muscle and the nerve cell body (Kristensson and Sjöstrand, 1972, Kristensson and Olsson, 1973). Watson (1974) suggested that "the axon tip is the dominant pole of the motoneurone and determines the metabolic state adopted by the cell", through the influence on the perikaryon of proteins ascending the axons. Thus, for somatopetal as well as for somatofugal transport, there existed both experimental and speculative grounds for examining the possible alterations induced by axonal injury.

SOMATOFUGAL TRANSPORT IN PROXIMAL PORTIONS OF DAMAGED AXONS

Early Attempts to Relate Axonal Transport to Regeneration

In 1942, Young mentioned the hypothesis "that the process of growth of the axon is essentially a flowing out

from the intact stump [of] the parent axon". The presence of a slow proximo-distal bulk flow of axoplasm in regenerating neurons was also considered by Lubińska (1964), along with mention of several studies reporting the bidirectional movements of axoplasmic inclusions in growing cultured neurons. On the basis of these observations, together with measurements of the rate of advance of growing axon tips, Lubińska suggested that the elongation of the fiber might be a reflection of the difference between the amount of material carried distally and the amount returning in the proximal direction. This concept represented a somewhat more realistic possibility than the previous version, as expressed by Young, which considered growth to be the result of material moving forth from the cell body simply in the distal direction.

Even so, the idea was still formulated in the most general terms, and when the first reports of experimental results began to appear, the findings were contradictory. Ochs, Kachmann and DeMyer (1960) injected radioactive phosphorus into both ventral horns of the spinal cord following unilateral crushes of cat sciatic nerve. By measuring the radioactivity in adjacent segments of the ventral root, it was concluded that the rate of axoplasmic flow was decreased below normal values between 3 and 15 days later on the side of the crush, but a comparable decrease was found on the uninjured side as well. In the period from 15 to 27 days, the values were closer to normal on the

uncrushed side, and covered an extended and less consistent range on the crushed side.

In 1968, Francoeur and Olszewski conducted an autoradiographic study of the fate of ^{3}H leucine labelled protein in normal and transected mouse sciatic nerves. Although no direct comparison of transport under the two conditions was given, synthesis of proteins, which were destined for transport down the axons, showed an initial reduction followed by an increase to above normal levels from about 1 to 30 days after transection.

The next attempt to investigate transport in regenerating nerve cells was that of Grafstein and Murray (1969), again employing tritiated leucine as the radiolabelled marker. Two different rates of transport were studied, referred to as slow and fast, but at a normal value of 40 mm/day, the "fast" rate was somewhat below the generally recognized range, even for goldfish optic nerve (Heslop, 1975). It was concluded that the slow rate was increased about three-fold (from 0.4-0.5 mm/day to 0.8-1.0 mm/day) beginning 6-8 days after section, reaching a maximum at about 2 1/2 weeks, and remaining elevated for several weeks after the regenerating fibers had reconnected with the optic tectum. The faster transport was judged to have approximately doubled its normal velocity at about 2 weeks after reconnection.

Kreutzberg and Schubert (1971b) studied transport of ^{3}H lysine labelled proteins in regenerating guinea pig hypoglossal nerves from 3 to 60 days following transection. They found that an accumulation of radioactivity just proximal to the transection, within the first few days, was later replaced by a significantly higher level of activity over the whole length of the nerve. This was interpreted as an increased amount of radioactive material entering the regenerating nerves by fast transport, but the authors were not convinced of any change in the velocity of the transport during regeneration. A peak of radioactivity appearing in association with the slow component was also interpreted as an indication of a redistribution rather than an increase in velocity of the labelled material.

In the same year another study (Carlsson, Bolander and Sjöstrand, 1971) was made of transported proteins, including choline acetyltransferase and acetylcholinesterase, in regenerating feline ventral roots up to 38 weeks postoperatively. Near the end of this period a decrease was found in the transport rates of both fast and slow ^{3}H leucine labelled proteins, with the fast rate dropping from 139 to 104 mm/day, and the average slow rate decreasing from 5 to 3 mm/day, in the regenerating roots. Initially choline acetyltransferase and acetylcholinesterase activity showed an increase proximal to the lesion and a marked decrease on the distal side, but by the end of the 36 weeks they had recovered to about 70% and 85% respectively, of control

values.

Problems of Interpretation and Experimentation

The picture that emerged from the first studies of axonal transport during regeneration was very inconsistent, with findings of increases, decreases, and no change, all being reported for both fast and slow transport. Part of the reason for this inconsistency was a lack of precision, both in the methods employed for measurement, and in the manner of analyzing the process. The method employed by Ochs, Kachmann and DeMyer (1960) for instance, was inexact, because its validity rested upon the assumption that efflux of the label from the injection site was linear. Subsequent work showed instead that the form of the outflow was exponential, and suggested that the apparent decrease during regeneration observed in the previous study may have been artifactual (Ochs, Dalrymple and Richards, 1962).

Lack of clarity in analyzing and interpreting results has been particularly evident in cases where conclusions about velocity are inferred from the accumulation of a label adjacent to a lesion. This problem was exemplified in the study by Grafstein and Murray (1969), where the property that was actually measured by the authors for the evaluation of fast transport was the time required for the radioactivity arriving in the optic tectum to reach one half of its peak value. Since this occurred in regenerating

nerves in half the time required by normal nerves, it was concluded that the velocity had doubled. However, since both the velocity and the quantity or density of material in transit co-determine the quantity of material that will arrive at any particular location, a change in accumulation is not necessarily an indication of any change in velocity.

In addition to the problem of distinguishing between velocity and measurements of which velocity is one component, a related difficulty has been the degree of ambiguity accompanying the use of the term "rate" in the literature. It has sometimes been used in the sense of distance per unit time (i.e. velocity), sometimes in the sense of quantity per unit time (i.e. flow rate), and sometimes it has not been clear which sense of the term was intended. It has therefore been necessary, in describing and discussing some of the literature, to employ such terms as "rate" in an imprecise sense; the general terms "increase" and "decrease" have also been used where the exact original meaning was not clear.

The more recent transport studies in regenerating neurons have in many cases avoided these problems, but there are further reasons that a consistent overall picture of axonal transport during regeneration will not be available until a number of specific aspects are more fully investigated. For instance, while variation in velocity is to be expected between the various classes of animals, there

are suggestions that the response of axonal transport to regeneration may differ substantially between different nerves of the same animal (Frizell and Sjöstrand, 1974c,d; Frizell, McLean and Sjöstrand, 1976).

Furthermore, the limitations of the radioactive labelling technique, upon which most of the research is based, have to be borne in mind. Since it is the amount of radioactivity, rather than the amount of proteins or other molecules, that is being measured, it is possible that apparent changes in the latter could be produced by variation in specific incorporation due to altered precursor pools (Frizell and Sjöstrand, 1974c). Another caution is suggested by the experiment of Cook and Whitlock (1975), who found that a progressing axonal crest of radioactive protein detected by liquid scintillation counting, was preceded by a faster component detectable only by autoradiographic technique.

DIFFICULTIES IN IDENTIFYING GENERAL RESPONSES DURING REGENERATION

For several reasons then, a unified view of the place of axonal transport in regeneration will only follow the assimilation of information gathered by a variety of different studies. Several other reports have been published since the original ones, and in general terms it would have to be said that an increase in velocity or amount of material transported (Grafstein and Murray, 1969; Gamache

and Gamache, 1974; Ingoglia, Weis and Mycek, 1975) appears to be more common during regeneration than a decrease (Carlsson, Bolander and Sjöstrand, 1971). More important however, is the fact that it has turned out to be very difficult to describe properly the response in general terms at all, since the majority of studies have produced mixed results of one form or another. In addition to the previously described studies of Ochs, Kachmann and DeMyer (1960), Kreutzberg and Schubert (1971b) and Griffin, Drachman and Price (1976), Frizell and Sjöstrand in particular have published a series of reports indicating that the responses observed differed between proteins and glycoproteins and between hypoglossal and vagus nerves.

In the first experiment (Frizell and Sjöstrand, 1974b), alterations in the transport of rapidly migrating proteins and glycoproteins were studied in the rabbit hypoglossal nerve. After 1 week of regeneration, the accumulation of choline acetyltransferase, acetylcholinesterase and ^{3}H leucine labelled proteins was reduced to the vicinity of 50-60% of control values, while the accumulation of ^{3}H fucose labelled glycoproteins was increased to 240% of normal. For the most part these alterations persisted at 4 and 6 week periods, although in lesser degree. The method used did not allow a distinction to be made between changes in velocity of transport or changes in the amount of material transported at the same velocity.

The second report (Frizell and Sjöstrand, 1974d) focused on the fast transport of glycoproteins and included observations in rabbit vagus as well as hypoglossal nerves. As before, a substantial increase of ^{3}H fucose labelled glycoprotein was observed in the regenerating hypoglossal nerves at 1 and 4 weeks. However, the transport velocity, which could be specifically calculated in this experiment, did not appear to be affected. Similarly, in the regenerating vagus nerve the transport velocity remained unchanged, but in contrast to the hypoglossal nerve, the amount of labelled glycoprotein accumulating proximal to the crush was significantly less at both 1 and 4 weeks. Possible reasons for the discrepancy were considered, but no clear explanation was available.

When transport of slowly migrating ^{3}H leucine labelled proteins was examined in the same two nerves (Frizell and Sjöstrand 1974c), the general pattern of greater activity in the hypoglossal and diminished activity in the vagus nerve was repeated. But in contrast to the stable velocities of rapid glycoprotein transport, adjustments in velocity were found during regeneration for slowly migrating proteins. The value of 4-5 mm/day, found in both normal hypoglossal nerves and in those contralateral to a nerve crush, approximately doubled, to about 9 mm/day after 9 days of regeneration. For technical reasons it was difficult to define the absolute velocity in the regenerating vagus nerve, but in all experiments at 9 and 10 days, the radioactive front moved

further distally in the contralateral than in the regenerating nerve. The amounts of labelled, slowly migrating, proteins also reflected the previous patterns of increase and decrease in the hypoglossal and vague nerves, respectively. The less vigorous response in the vagus nerve was attributed to a less developed or less successful attempt at regeneration, although labelled proteins were transported into the growing fibers distal to the crush in both nerves. Whatever the explanation, it was demonstrated that substantial differences existed during regeneration between the hypoglossal and vagus nerves of the rabbit, in terms of both velocity and amount of slowly migrating proteins, and in the amount of rapidly migrating glycoproteins. In addition, a different response was exhibited by proteins than by glycoproteins in the regenerating hypoglossal nerve. Overall, this series of experiments provided an indication of the kinds of variability that may be expected to occur in the transport system during regeneration. Evidently such factors as the specific nerves involved, the particular chemical components being transported, and the length of time elapsed, will play a large part in determining the exact response in any given situation.

If the idea of an overall increase or decrease in axonal transport accompanying regeneration is too simplistic, are there any general statements which are likely to be applicable? The concensus of evidence is that

the velocity of rapid axonal transport remains unchanged, while any differences in material circulating are effected mainly by changes in the amount of material contained in the stream (Ochs, Dalrymple and Richards, 1962; Kreutzberg and Schubert, 1971b; Frizell and Sjöstrand, 1974b,d; Ingoglia Weis and Mycek, 1975; Griffin, Drachman and Price, 1976; Bisby, 1977), and this possibility has been advocated by Ochs (1972b, 1975). On the other hand, the evidence for net increases or decreases in the actual amount of material transported is still very inconsistent.

COMPARISON WITH TRANSPORT DURING EMBRYONIC AND POSTNATAL DEVELOPMENT

Given that considerable uncertainty presently exists concerning the characteristics of axonal transport during regeneration, it may be instructive to examine what is known of the process during the initial embryonic and postnatal development of nerves. Because of the similarity between regeneration and the original developmental outgrowth of nerve fibers, the properties of axonal transport during prenatal and early postnatal in vivo development might provide a useful perspective on alterations to be expected in the regenerating axon. Such a comparison is further encouraged by the somewhat greater consistency to date among studies of transport during development than during regeneration. Most of these experiments are based upon the injection of radioactive precursors into regions occupied by

nerve cell bodies, and subsequent detection of the transported label by liquid scintillation counting or autoradiography.

There is good agreement on the development of rapid transport of proteins in avian embryos and amphibian larvae. Bondy and Madsen (1971), Marchisio et al (1973), Sjöstrand, Karlsson and Marchisio (1973), Gremo and Marchisio (1975a,b) and Marchisio, Gremo and Sjöstrand (1975) all reported increases with age in fast axonal protein transport in the optic nerves of chick embryos. Crossland et al (1974), comparing protein transport velocities in embronic and hatched chicks, found an increase from approximately 92 mm/day in the 5.5 day embryo to about 200 mm/day at 5-6 weeks after hatching, and in frog larvae (stage VI) calculated a velocity which was lower than that in the adult (Edström and Hanson, 1973a).

Discrepancies again appeared in connection with rapid protein transport in neonates. The observations of Hendrickson and Cowan (1971) indicated a continuation of the increase in transport reported for earlier stages. They found a continual increase in the velocity of rapid protein transport in newborn rabbits from 6 days to 4 weeks of age, at which time the velocity attained a fairly constant value which continued into adult life. On the other hand, the studies of Gremo and Marchisio (1975b) and Marchisio, Gremo and Sjöstrand (1975) revealed a decline in the rapid

transport of proteins in the chick optic nerve, beginning at about the time of hatching and leveling off at adult values thereafter. These two sets of observations are not necessarily contradictory however, because Hendrickson and Cowan dealt exclusively with measurements of transport velocity, while in the latter experiments transport was expressed in terms of the proportion of injected precursor that was exported to the periphery within a given period of time. It is possible that the velocity continues to rise or remains constant while a declining amount of material is being loaded onto the transport system.

General agreement is again the rule when the studies of slow protein transport during development are considered. Marchisio et al (1973), although they did not analyze slow protein transport in detail, did calculate its velocity in the chick optic nerve, and compared it to other reported velocities both before and after hatching. The comparison indicated that there was a continuous decrease in the velocity of slow axonal transport throughout embryonic and early post-embryonic development. The same conclusion was expressed and discussed by Marchisio and Sjöstrand (1972).

Slow protein transport under postnatal and mature conditions was compared by Droz and Leblond (1963), Droz (1965) and Lasek (1970a). The former two reports drew attention to the consistently higher velocities observed in the younger animals. Average values differed by a factor of

about 2-3, and the same approximate ratio was reported by Lasek (1970a), who found velocities in kitten sciatic nerve to be 2-3 times greater than those in the cat. Hendrickson and Cowan (1971) reported a postnatal decrease in slow protein transport in rabbit optic nerves from approximately 5 mm/day at the end of the first week to about 2 mm/day around 4 weeks of age. The change was described not as gradual but rather rapid during the fourth week, and the authors considered that this period might coincide with the establishment by the retinal ganglion cell axons of definitive synaptic connections in the superior colliculus.

While no slow rate has been described for embryonic or postnatal transport of glycoproteins, rapid glycoprotein transport appears to follow a similar pattern to that of the proteins. In embryonic chick optic nerves, an increase in rapid glycoprotein transport as a function of age was observed by Bondy and Madsen (1971), Marchisio et al (1973), Sjöstrand, Karlsson and Marchisio (1973), Gremo, Sjöstrand and Marchisio (1974), Gremo and Marchisio (1975a,b) and Marchisio, Gremo and Sjöstrand (1975). In the newly hatched chick, the proportion of injected precursor exported along the axons was found to decrease during the first few weeks (Gremo and Marchisio, 1975b; Marchisio, Gremo and Sjöstrand, (1975) but as before, this measurement does not necessarily reflect changes in velocity.

The overall results to date of research on axonal

transport in developing neurons have been consistent enough to establish certain general trends. Rapid protein and glycoprotein transport has consistently been found to increase with age during middle and late embryological development, and during the first few weeks in the newborn, tends to adjust to stable adult levels, although the direction in which the adjustment occurs is presently unclear. Slow transport of proteins has consistently been observed to undergo a continual decrease in velocity from its earliest detection in the emrbyo to its final minimum level in the matured nerves of the adult.

The general hypothesis could be suggested that slow protein transport is primarily associated with axonal growth, particularly in length, and that the rapid phase is associated mainly with neurotransmission (or neurosecretion) and steady state metabolism. This possibility would lead to the prediction of relatively high rates for slow transport during early development and low rates during steady state conditions, and to initially low rates for rapid transport increasing to higher levels in fully developed nerves and tracts. These predictions are largely borne out by the experimental results.

The hypothesis would also have implications for protein transport in regenerating neurons, under which conditions growth again assumes a dominant, and synaptic transmission a diminished, role. In these circumstances, an increase in

slow transport and decrease in rapid transport might be anticipated. Any such general changes during regeneration could theoretically occur either through alterations in transport velocity or through changes in the amount of material "loaded" onto the transport system. In the studies of transport during development, most of the changes reported were in velocity, a situation which appears to be at odds with the opinion that velocity may be the one stable characteristic of transport in the studies of regenerating neurons. However, the fact that the developmental changes appeared primarily in velocity was usually attributable to the design of the experiments, and did not preclude the possibility of concomitant changes in the quantities of materials involved. It may be that adjustments during development can be effected through alterations both in velocity and in quantity of material per unit volume, while changes in the more mature neurons during regeneration might be possible only through alterations in the latter variable.

SOMATOPETAL TRANSPORT IN PROXIMAL PORTIONS OF DAMAGED AXONS

Research on somatofugal transport has overshadowed investigations of somatopetal transport in damaged and regenerating neurons, as was the case in normal neurons, again, largely because of the greater ease of labelling for material moving in the distal direction. However if the chromatolytic reaction is to be triggered (Cragg, 1970; Ochs, 1972b; Kristensson and Olsson, 1973, 1974, 1975, 1976;

Halperin and La Vail, 1975; Bisby and Bulger, 1977), or if the regenerative response is to be regulated (Kristensson and Sjöstrand, 1972; Kristensson and Olsson, 1973, Watson, 1974, Frizell, McLean and Sjöstrand, 1976; Bulger and Bisby, 1978), by ascending chemical influences in the axons, an understanding of somatopetal transport in damaged axons will be important. Somatopetal transport has similar implications for control of the initial development of nerve fibers and possibly of synaptic connections (Gremo and Marchisio, 1975b).

Before any direct experimental evidence existed for somatopetal transport in regenerating axons, Watson (1968a) observed that the nucleolar response to axonal injury began and declined earlier, the closer the injury was to the cell body. Although there were other indications that these changes were not induced simply by removal of contact between the neuron and its motor terminal, the temporal dependence of the reaction on the distance from the injury was consistent with the idea of its initiation or regulation by some kind of message moving somatopetally along the axon.

Kreutzberg and Schubert (1971a), in a study of volume changes in axons central to the level of transection, expressed the opinion that some aspect of the transport and volume changes was likely to account for the neuron's ability to respond appropriately to the peripheral requirements.

Direct evidence of somatopetal transport in injured and regenerating axons appeared the following year. Kristensson and Sjöstrand (1972) found that Evans blue labelled albumin (EBA) was taken up by nerve terminals and transported to the cell bodies by regenerating rabbit hypoglossal neurons from 1 to 4 months after crushing. Litchy (1973) and De Vito, Clausing and Smith (1974) demonstrated an uptake and somatopetal transport of another exogenous protein tracer, horseradish peroxidase (HRP), from intact terminals and cut ends of amphibian and mammalian nerves. HRP was also shown by Halperin and LaVail (1975) to be taken up at damaged retinal ganglion cell terminals in the chick retina and carried to the cell bodies in the isthmo-optic nucleus.

In two of these experiments, the patterns of arrival of the tracer proteins at the cell bodies of the injured neurons were compared to those of uninjured neurons, and were found to be distinctly different. Kristensson and Sjöstrand (1972) found that, while EBA accumulated in the nerve cell bodies of intact control neurons within 10-24 hours after intramuscular injection, none appeared in the cell bodies of crushed neurons for over 3 weeks. An increased intensity of tracer was seen in the injured cells relative to the control side from 35 to 48 days after the crush, and by 80 to 120 days the difference between sides was no longer detectable. Halperin and LaVail (1975) examined the accumulation of HRP over a shorter time period, immediately following its injection into the vitreous body

of chick eyes. The tracer first appeared in the bodies of the uninjured cells 3.5 hours later, whereas the accumulation began at 4-6 hours in the injured neurons. Between 6.75 and 18 hours, the injured cells accumulated significantly more HRP than normal cells, but from 24 to 72 hours later they contained significantly less tracer. The differences were considered to be mediated by variations in the uptake of HRP by the injured nerve terminals rather than by any change in the velocity of transport.

Somatopetal transport of radioactively labelled endogenous proteins and glycoproteins was documented by Sjöstrand and Frizell (1975) and Frizell, McLean and Sjöstrand (1976), using a method based upon the reversal of somatofugally transported material at natural and artificial nerve endings (see Chapter 5). Comparison of accumulations on the distal sides of ligatures on unoperated and regenerating nerves at 1 and 5 weeks indicated the presence of a rapid somatopetal transport of proteins and glycoproteins in both regenerating hypoglossal and vagus nerves, which was greater than that present in the control nerves. Further evidence of protein reversal and retrograde transport in regenerating nerves was reported by Bulger and Bisby (1978).

It has been adequately established by these studies that somatopetal transport continues to operate in damaged and regenerating axons. Both foreign and native substances

can be carried to the cell bodies by the system, and both increases and decreases in amounts of material conveyed are possible, depending on the particular circumstances. It is therefore possible for the cell bodies to receive information concerning conditions affecting the axons through both qualitative and quantitative variations in material arriving by somatopetal transport.

AXONAL TRANSPORT IN REGENERATING SPROUTS

Transport in regenerating axonal sprouts has been studied recently. Griffin, Drachman and Price (1976) measured the same velocity (383 ± 33 mm/day) for fast somatofugal transport of ^{3}H leucine labelled proteins in both the central stumps and in the regenerating sprouts of rat sciatic motor nerves. No impediment was apparent at the level of the axotomy, and autoradiography indicated that the labelled proteins were accumulating in the endings of the sprouts. McLean, Frizell and Sjöstrand (1976) also reported transport of radioactive proteins through the crush zone and into the newly grown motor and sensory fibers of the rabbit vagus nerve.

It appears then that the process of rapid somatofugal transport in regenerating sprouts resembles that in the normal axon, and that it probably functions to convey newly synthesized materials to the growing axon tips. Although the labelled protein was arrested at the crush zone in vagal fibers incubated in vitro rather than in vivo following

precursor injection (McLean, Frizell and Sjöstrand, 1976), this failure was likely to have been an indirect effect on the transport system of some other abnormality existing under the in vitro conditions.

STATEMENT OF OBJECTIVES

The objectives of the work reported in this thesis are:

1. To develop the particular perspective of one of the newer approaches to axonal transport: the optical detection of particulate material in axons isolated from mature animals.
2. To define the properties of the organelle transport observed by this technique in normal axons.
3. To investigate whether the transport is altered in regenerating axons.
4. To attempt to account for the organelle transport observed in interrupted and regenerating axons by investigating events at the ends of the crushed fibers.

CHAPTER 2

OPTICAL DETECTION OF ORGANELLE TRANSPORT IN NORMAL AXONS

INTRODUCTION

The work reported in this chapter was among the earliest efforts to describe the movements of organelles which had only recently been shown to be detectable by optical methods in living mature nerve fibers (Smith, 1971, 1972a, 1973; Kirkpatrick, Bray and Palmer, 1972; Kirkpatrick and Stern, 1973). In myelinated axons isolated from adult Xenopus laevis, three groups of organelles differing in appearance or behavior, were detected. This work is described in the present chapter; the results have also been reported in a paper (Cooper and Smith, 1974).

METHODS

The animals used were adult male and female Xenopus laevis (African clawed toad). These animals were in good nutritional condition and were kept in tanks of water at room temperature. In most experiments the animals were pithed, but in some cases, as for the isolation of parts of the spinal root system, they were anaesthetized by submersion in a 2% solution of urethane (ethyl carbamate). Nerves were dissected free from the animal, and single myelinated nerve fibers were subsequently isolated, under a Ringer solution of composition (mM): NaCl, 112; $NaHCO_3$, 2.5; KCl, 2.5; $CaCl_2$, 2.0. The Ringer solution was gassed with

95% O_2 and 5% CO_2 before use. All experiments were conducted at room temperature (20-22°C).

Most of the observations were on nerve fibers which were 10-20 µm in diameter. These were isolated at the centre of a 5 cm length of sciatic nerve taken from the hip to just below the knee joint. Approximately 50 such preparations were used. In a small number of cases fibers from parts of the eighth spinal roots and from identified sensory and motor nerves in the leg were used: ventral root fibers, three experiments; dorsal root fibers central to the dorsal root ganglion, five experiments; dorsal root fibers distal to the ganglion, three; sensory nerve fibers to the knee joint and to leg skin, three; large diameter motor nerve fibers to the iliofibularis muscle (identified as in Smith and Lännergren, 1968), four.

Isolated nerve fibers were mounted for observation in a chamber made from a microscope slide (Figure 2.1). Two longitudinal grooves 7 mm wide were milled with a diamond cutter in each half of the slide to a depth of half the thickness of the slide. A central table about 2 mm wide separated the two grooves. The undissected portions of the nerve rested in each groove while the table supported the single nerve fiber. The top surface of the slide was lightly greased with petroleum jelly to support and seal a cover slip. This chamber allowed very close apposition of the cover slip to the isolated part of the nerve fiber without

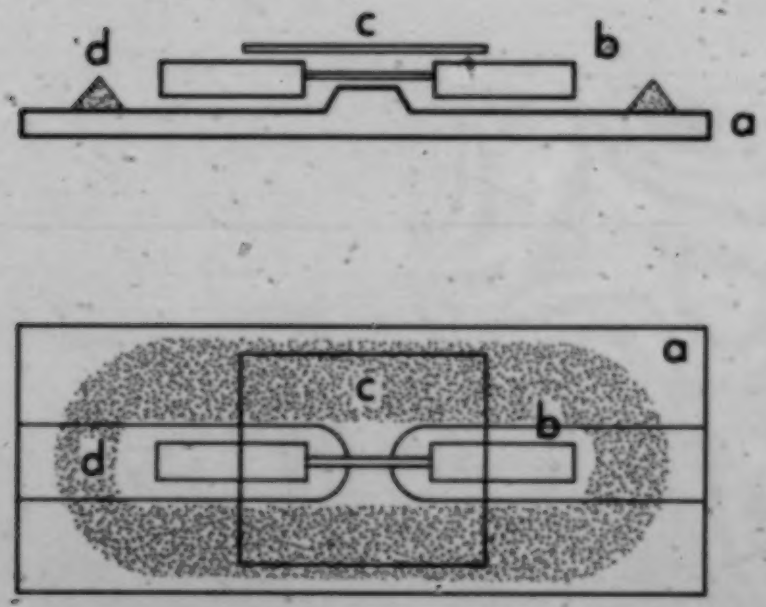


Figure 2.1. Diagrammatic top view, below, and section, above, of the viewing chamber. A microscope slide (a) had two longitudinal grooves 7 mm wide milled to half the thickness of the slide. At the center of the slide the two grooves were separated by a table 2 mm wide which supported the single nerve fiber. The dissected piece of nerve is shown in place (b). The nerve was immersed in a Ringer solution (not shown). A cover glass (c) was placed over the preparation. A layer of petroleum jelly (d), indicated by the stipple, prevented the cover glass from touching the single nerve fiber and also sealed the cover glass to the slide. The petroleum jelly at the ends of the slide prevented the Ringer solution from leaking off the slide.

crushing those parts remaining in the undissected nerve bundle. Fresh Ringer solution could be perfused through the chamber by dripping it into one end of the central groove and removing the solution emerging at the far side of the cover slip.

Organelles within the axoplasm of the single nerve fibers could be detected using either Nomarski optics or darkfield optics (Smith, 1971, 1972a). In most cases darkfield illumination was the more useful. A Zeiss Ultra darkfield condenser was used with a 100X planachromatic objective at a numerical aperture of 0.9-0.8. The light source was a quartz-iodide lamp. Heat filters in the optical path prevented unintentional heating of the preparation.

The motion of organelles within the axoplasm was recorded either on motion picture film (see Plate 2.2) or by noting the times at which organelles crossed the fine lines of an ocular grid. Motion pictures of the darkfield image were taken at 3 or 4 frames/sec with Kodak 2479 RAR 16 mm film. The camera, mounted to a rigid frame separate from the microscope, was driven by a servomotor via a flexible coupling. Nomarski images could be filmed at 12 frames/sec with Kodak 4X film. The motion picture records were viewed using a frame counting analysis projector, and the images were either measured directly, or in a few cases were digitized and subsequently analyzed with computer programs developed by McLeod (Smith, Koles and McLeod, 1979).

Distances were calibrated against the image of a 10 μm grid. With the alternate method of recording organelle motion an ocular grid was used having 20 lines spaced either 5.00 μm or 3.13 μm apart. The lines were arranged to fall across the nerve fiber at right angles to its axis. Each time an organelle crossed one of the lines the observer closed a microswitch causing a line to be drawn across a strip chart. The time axis on the strip chart record was calibrated with a 1 Hz square wave. This method had the advantage that organelles could be followed for distances of up to 100 μm by adjusting the focus slightly. On the other hand, motion picture film in addition to providing a permanent record of typical events also captured rare events. The results of either of the methods allowed distance-time plots to be constructed for the motion of individual organelles. Straight lines were fitted to these plots by a least squares method. Except where noted in the text, sufficient data were gathered so that the correlation coefficient between the variables (distance and time) was at least 0.98. The slopes of the lines then gave mean velocities for individual organelles. The velocities so obtained represented transport velocities in the axial direction of the nerve fiber. Except in unusual cases this value was very close to the speed of the organelle along its trajectory; markedly curving trajectories through the axoplasm were not common.

Plate 2.1. Darkfield photomicrographs of a myelinated axon showing the appearance of organelles within the axoplasm. The two photographs A and B were taken at slightly different positions of focus to demonstrate the large number of organelles which may be detected. In each photograph the bright horizontal bands at top and bottom were caused by light scattered from myelin. In A, round organelles of various diameters may be seen (arrows), as well as axially oriented rod-shaped organelles of various lengths. The very long rod-shaped image between the arrows was caused by the overlapping images of two separate rods. In B the focus was shifted slightly. Images of the two round organelles at the upper left, arrows in A, are still present, but organelles not detected in photograph A may now be seen. A small round organelle is closely associated with one of the rods (arrow). The photographs were taken after organelle motion had been stopped by perfusing the viewing chamber with a buffered 5% glutaraldehyde solution. Scale bar, 10 μm.

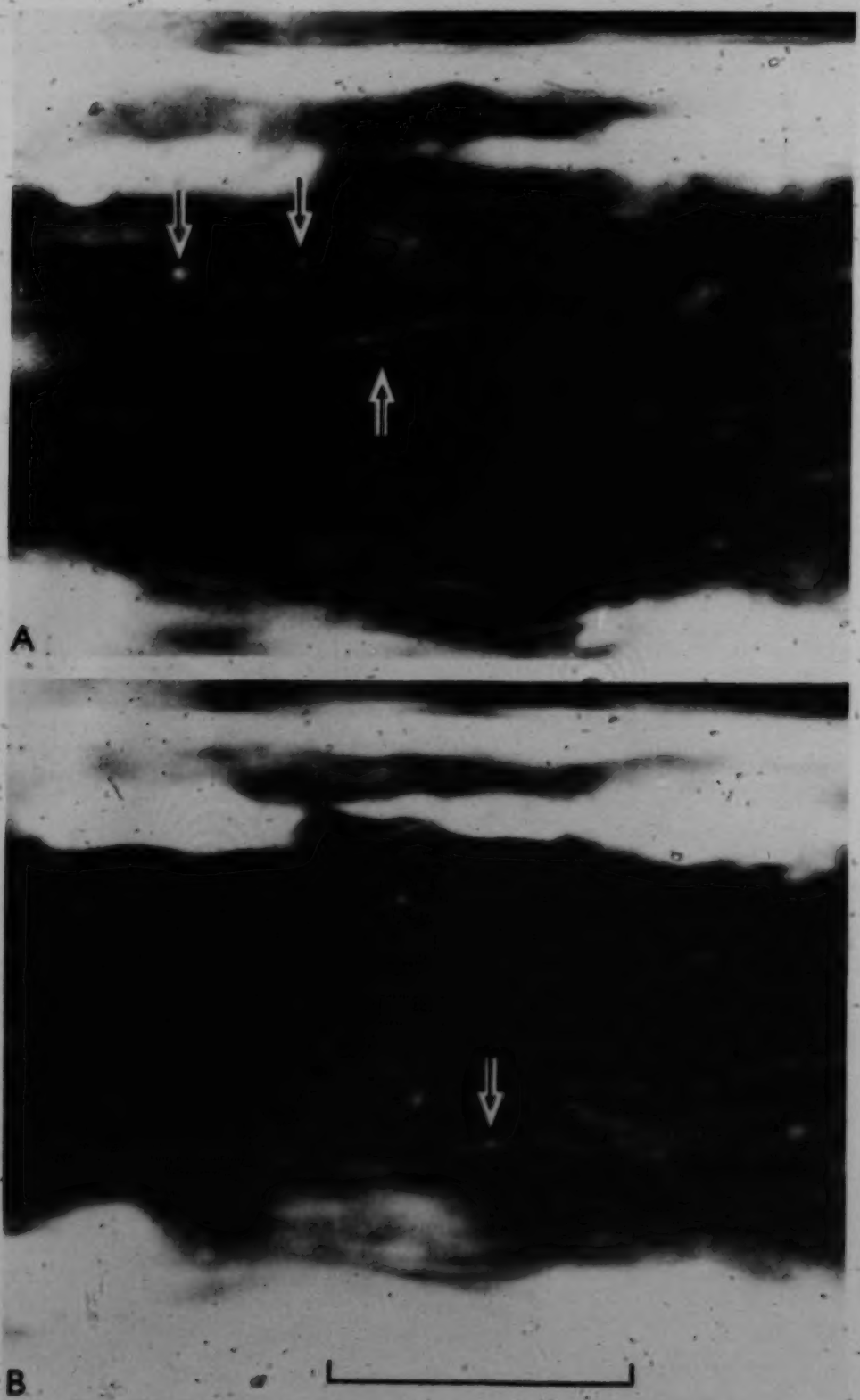
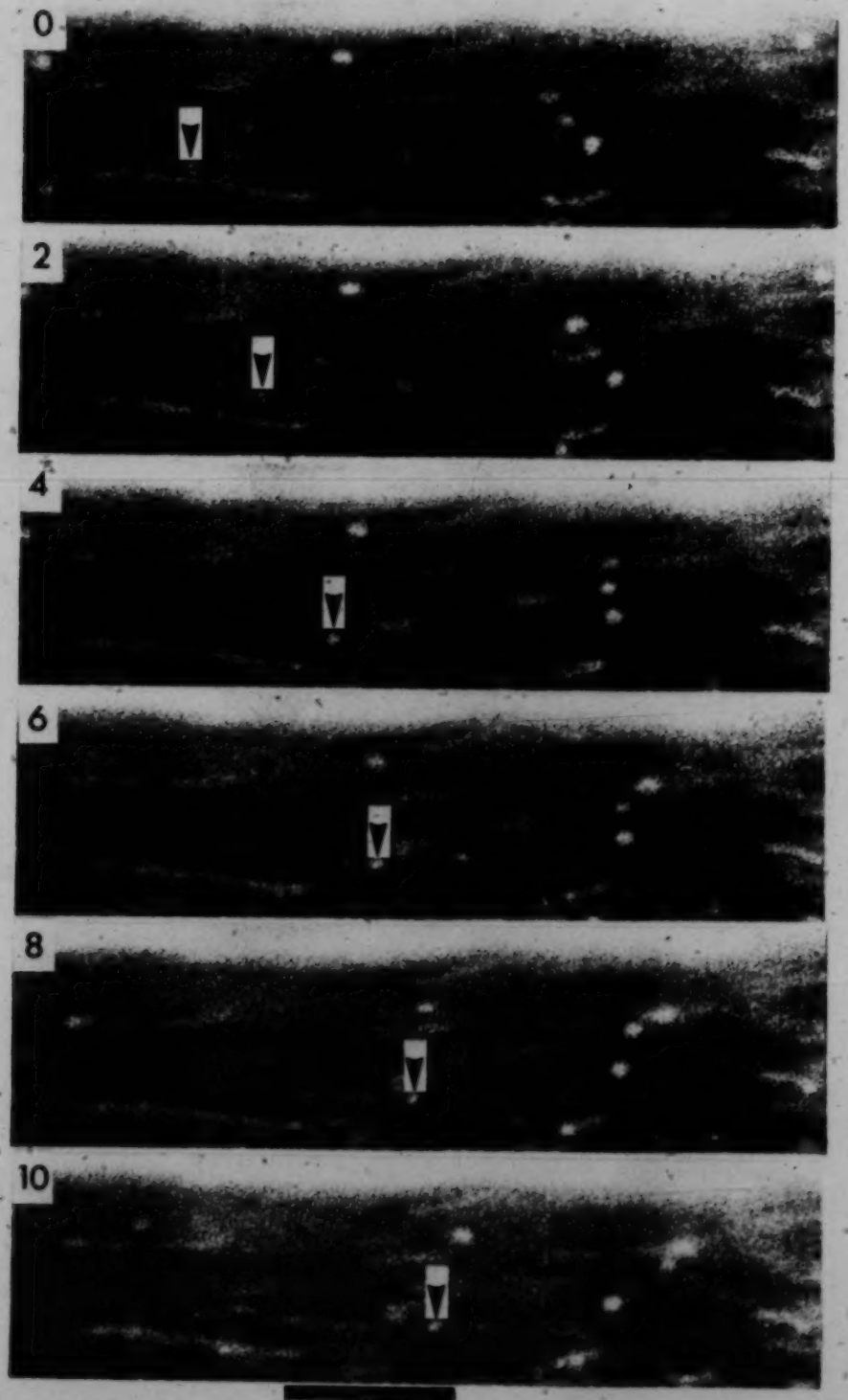

A
B

Plate 2.2. Photograph of six frames from 16 mm motion picture records used in analysis of particle movements (see Methods). Sequence shown covers a 10 second period at 2 second intervals. (Motion picture films were exposed at 3 frames per second). Stationary round and rod-shaped images are evident, as well as both somatopetally (arrows) and somatofugally moving round images. Scale bar, 5 μm.


0
2
4
6
8
10

RESULTS

GENERAL OBSERVATIONS

Darkfield microscopy (Plates 2.1 and 2.2) revealed in all axons, including the known sensory and motor fibers, three groups of organelles. Particles with round images (diameter 0.2-0.5 µm) or slightly elliptical images (0.2-0.3 x 0.5 µm) moved at about 1 µm/sec in the somatopetal, or, in the sciatic nerve, proximal, direction. A similarly shaped group of organelles with images 0.2-0.3 µm in diameter moved at a similar rapid rate in the opposite direction, away from the cell body. The motion of organelles in these two groups was irregular in a manner that is termed saltatory (Rebhun, 1972; Berlinrood, McGee-Russell and Allen, 1972). Each particle appeared to move independently of the others and both streams of particles occupied the whole axoplasmic space within the internodes. While any given particle might hesitate in its course, and some even reverse direction for a few micrometers, no particle changed its direction of movement entirely. Two differences were noted between these two groups of organelles: the somatopetally moving group contained about ten times as many detectable organelles as did the somatofugally moving group; the former group also contained the largest organelles.

The third group of organelles consisted of rod-shaped bodies approximately 0.2-0.3 µm in diameter with lengths ranging from 1 to 8 µm. These were usually stationary in the

axoplasm although occasionally a rod was observed with a saltatory motion similar to that of the round organelles.

In the following parts of this report these observations will be treated more fully. A detailed analysis of the erratic motion of individual organelles will, however, not be undertaken here; consequently the use of the descriptive term saltatory requires some justification. The term is applied to the rapid (in the order of microns per second) and irregular, or jumping, motion of intracellular particles that is not likely to be a consequence of thermal agitation (Rebhun, 1972). In this work the discontinuous jumping motion was most obviously present in aging preparations (see section on status of the preparations). However, this kind of jumping motion also occurred in fresh preparations, as shown in Figure 2.8, open circles, and Figure 2.9. Most of the round organelles in fresh preparations did not show such clear saltatory motion; in these the jumps were more closely linked giving rise to an almost continuous motion which took place in what appeared to be major periods of acceleration and retardation of about 10 μm length (Figure 2.4). Thus it seemed that the motion of all the organelles observed in this study might reasonably be described as saltatory. Coarse sampling of the trajectories of particles did not, in general, reveal these fluctuations in velocity (see Figure 2.5).

STATUS OF THE PREPARATIONS

Most of the results were obtained from observation of the axoplasm at locations which were about 2.5 cm distant from the cut ends of the fiber. Effects of severing the axons on the motion of the organelles cannot be discounted; there obviously must be a marked effect close to the site of nerve section. It was not possible prior to the development of more specialized techniques (Chapter 5) to view these effects very close to the cut ends, owing to the poor optical conditions there. In two cases organelle movements were observed 1 mm from the cut end between 20 and 35 minutes after the cut was made. The motion of these organelles did not differ in any detectable way from that usually observed at the center of a 5 cm length of sciatic nerve. On the other hand, damage at or very close to the portion of nerve being observed was immediately apparent; the myelin became wrinkled and particle motion in the direction of the longitudinal axis of the fiber ceased and was replaced by a Brownian motion. Subsequent observations (reported in Chapter 5) made possible by alterations in experimental technique have confirmed the opinion that effects at the cut ends of the nerve fibers were unlikely, within the existing time and distance constraints, to have had any direct influence on the behavior of the organelles at the observation site in the center of the nerve segment.

The length of time that the preparations could be considered to be in a steady state varied from nerve to

nerve. Two types of changes were characteristic of an aging preparation: the number of organelles passing a given diameter of the axon per unit time decreased, and later the motion of individual organelles altered. For example, a typical preparation had a mean of 8.2 particles/min crossing a given diameter of the axon in one focal plane during the first hour of observation. At the end of the second hour the number was 8.4 particles/min. Thereafter the number of moving particles declined: 5.2 per minute at 3 hours and 1.6 per minute at 4 hours. Alterations in the motion of the particles became noticeable at later times; the pause between saltatory jumps became very long for a large number of particles. Although normal motion of individual organelles was observed in some axons up to 14 hours after the nerve was removed from the animal, the measurements and observations in this chapter were made during the first 2 hours, while the preparations were in the steady state described above.

MOTION OF THE ROUND ORGANELLES

Direction of Motion

The majority of round organelles detectable with either Nomarski or darkfield optics moved toward the soma of the nerve cell. In axons from the sciatic nerve, ventral roots, and dorsal roots distal to the dorsal root ganglion, about 10 times as many particles moved proximally (towards the

spinal cord) as distally (Figures 2.2 and 2.3). In axons from the dorsal roots central to the dorsal root ganglion most of the particles moved away from the spinal cord and toward the ganglion. Thus, the terms somatopetal and somatofugal, while cumbersome, are appropriate to describe the directions of movement of the larger and smaller groups of particles.

An analysis of darkfield motion pictures representing continuous periods of observation of 25 minutes for each of three sciatic nerve fibers gave a mean value for the number of particles crossing a diameter of the axis cylinder of 8.99 particles/min per 10 μm diameter in the somatopetal direction, and 0.57 particles/min per 10 μm diameter in the somatofugal direction (ratio approximately 16:1). Fibers from the dorsal roots central to the dorsal root ganglion contained fewer moving particles. Motion pictures of two of these fibers gave mean values of 2.47 particles/min somatopetally and 0.25 particles/min somatofugally for 10 μm widths of axon. Here the ratio of somatopetally to somatofugally moving particles was close to 10:1.

Velocities

Figure 2.4a exemplifies the generally linear and axial trajectory of a particle travelling in the somatopetal direction in a sciatic nerve fiber. Figure 2.4b shows its typical time course of displacement in the X-direction. The

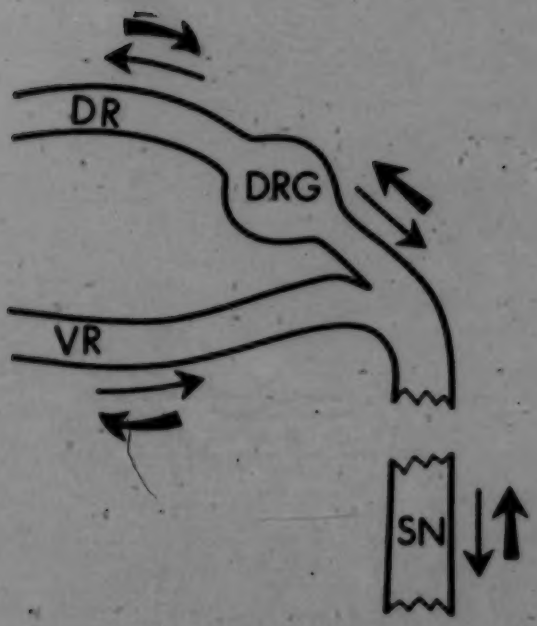


Figure 2.2. Diagram of the 8th spinal roots and the sciatic nerve to show the directions in which most particles (thick arrows) and fewest particles (fine arrows) were seen to move. In all cases most particles travelled somatopetally. The ratio of the numbers of particles travelling in the two directions was at least 10:1 at all locations, but fewer moving particles were detected in the dorsal roots central to the dorsal root ganglion than at the other locations. DRG, dorsal root ganglion. DR, dorsal root. VR, ventral root. SN, sciatic nerve.

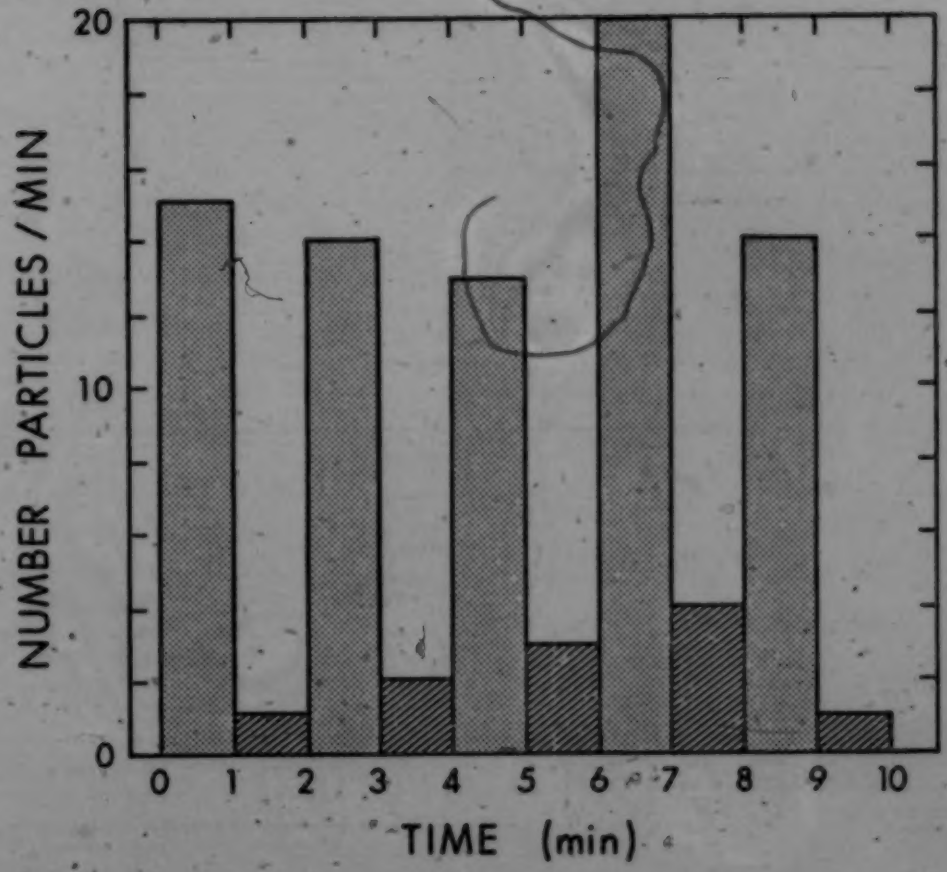


Figure 2.3. Histogram showing the numbers of particles which crossed a diameter of the axis cylinder in the somatopetal direction (light bars) and in the somatofugal direction (dark bars) in alternate 1 minute intervals. Diameter, 9.5 µm. Sciatic nerve fiber.

Figure 2.4. Details of the motion of a particle travelling in the somatopetal direction (a,b,c and d) and of a particle travelling in the somatofugal direction (e,f,g, and h). Parts a and e show the trajectory taken by each particle; in each case the X axis represents the longitudinal axis of the nerve fiber, and the values of X increase in the somatopetal (proximal) direction. Parts b and f show (points) the X distance travelled as a function of time; the straight lines were fitted in the least squares sense and have slopes representing particle velocities of: b, 0.94 μm/sec (somatopetal), and f, 0.85 μm/sec (somatofugal). Parts c and g show the velocity of each particle as a function of distance; in g the particle, travelling from higher to lower values of X, is assigned negative values of velocity. Parts d and h show particle velocity as a function of time.

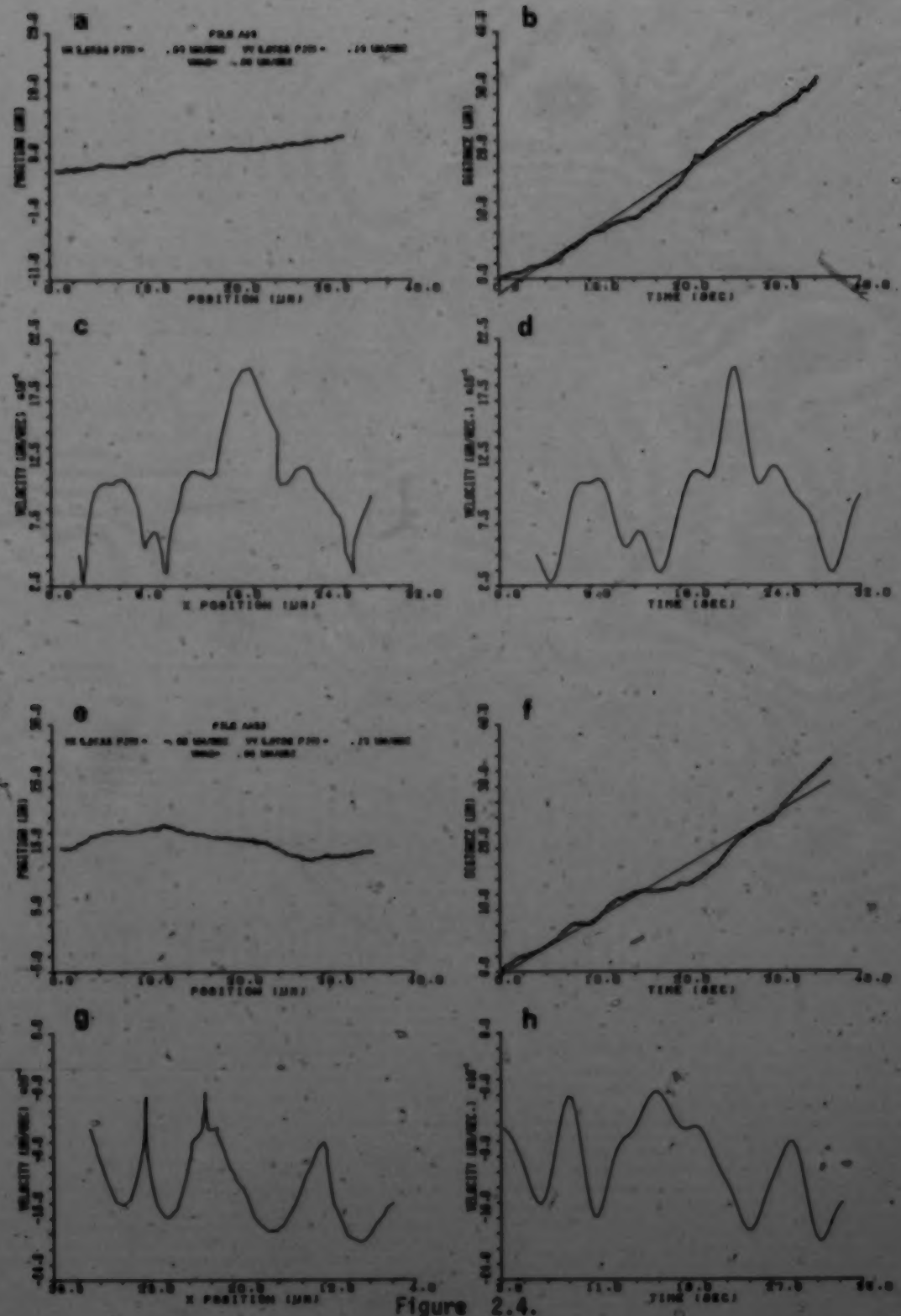


Figure 2.4.

velocity of the particle (Figure 2.4d) was derived from the measurements of Figure 2.4b and was from moment to moment quite variable. The visible movements were characteristically irregular, consisting of what are assumed to be closely linked saltatory jumps. This property of the movement was plainly evident during observation, and is demonstrated more clearly in Figure 2.4c where velocity is expressed as a function of distance rather than time. Figure 2.4e,f,g and h indicates that these properties were basically similar for particles moving in the somatofugal direction. The question was not pursued of whether these small scale variations in velocity were real or were caused by errors in measurement; the purpose of Figure 2.4 is to demonstrate that the motion of the particles could be described in terms of a mean velocity of about 1 µm/sec. Distance-time plots for three representative particles travelling in the somatopetal direction are shown in Figure 2.5a and for the somatofugal direction in Figure 2.5b. Each set of points is fitted with a least squares straight line whose slope represents the mean velocity of the particle. Figure 2.6a is a histogram of 150 such mean velocities for somatopetally travelling particles. Figure 2.6b is a similar set of values for 50 somatofugally travelling particles. The means and standard deviations for these two sets of determinations are 0.98±0.37 µm/sec and 1.32±0.49 µm/sec respectively.

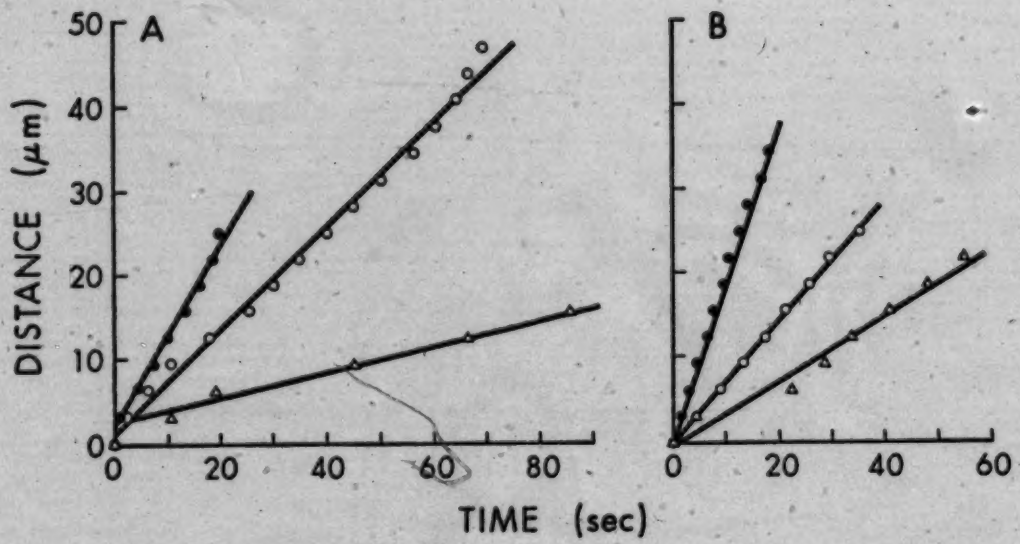


Figure 2.5. Example distance-time plots for three particles travelling somatopetally (A) and three particles travelling somatofugally (B). Each set of values is fitted with a straight line whose slope describes the average velocity of the particle.

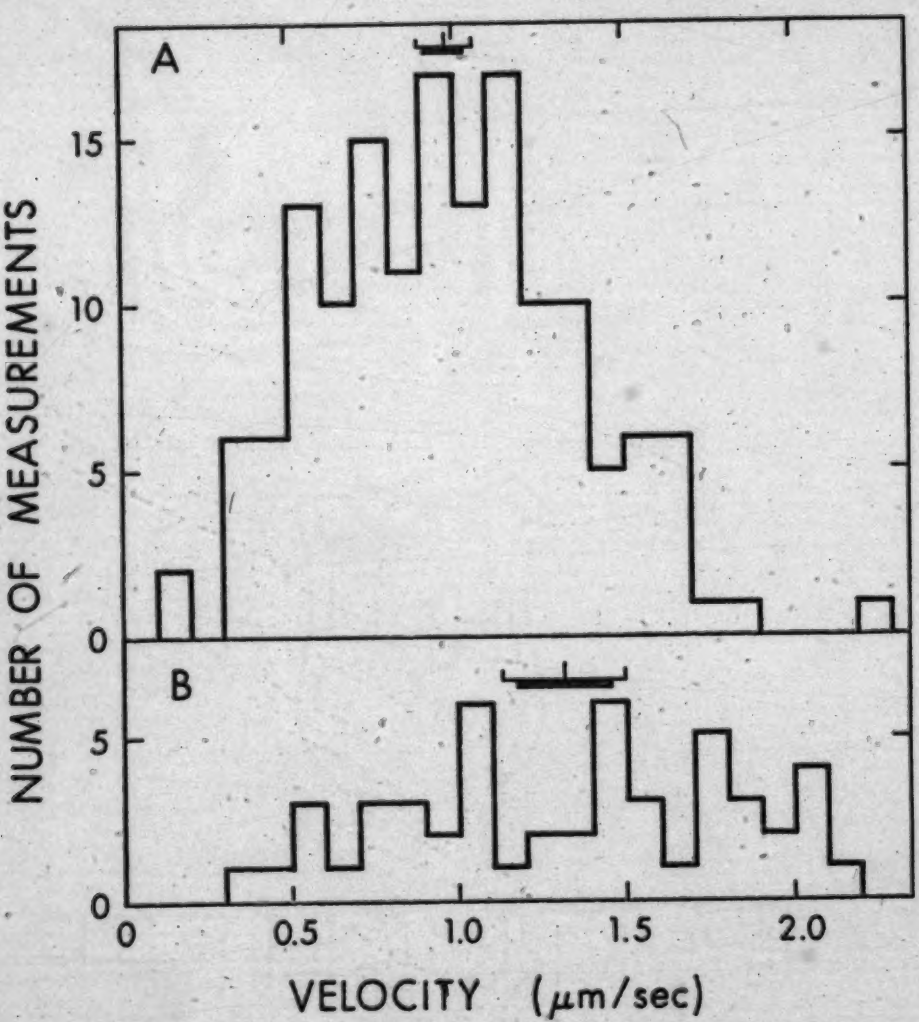


Figure 2.6. Histograms of the average velocities of individual particles in sciatic nerve fibres. A, a collection of 150 velocities of somatopetally travelling particles. B, 50 velocities of somatofugally travelling particles. Above each distribution are indicated the position of the mean of the distribution and 99% (fine lines) and 95% (thick bar) confidence intervals for the mean.

Motion across Nodes of Ranvier

Measurements of the velocities of organelles in the internode of the myelinated nerve fiber can be extrapolated to long distances of the axon only if the organelles are not greatly impeded at the node of Ranvier. Observations of organelles at nodes of Ranvier were very difficult owing to the light scattered by the edges of the myelin. Observation by darkfield microscopy was successful once and four preparations were observed with Nomarski optics. Examples of trajectories followed by a number of organelles crossing the node in the somatopetal direction in one preparation are shown in Figure 2.7. The envelope of the trajectories entering this node followed the shape of the nodal constriction but particles tended to remain centrally placed in the axoplasm on leaving the node. Some particles stopped on entering the node (filled circles, Figure 2.8) and continued their progress across the node after variable periods of time. A particle occasionally remained arrested at the inlet of a node for several minutes. Other particles (open circles, Figure 2.8) traversed the node without any apparent hindrance. The two curves of Figure 2.8 demonstrate that at the node, as elsewhere in the axon, the motion of any one particle appeared to be independent of that of its neighbours. The one successful experiment in observing the node by darkfield illumination showed that round organelles crossed the node somatofugally in a similar manner.

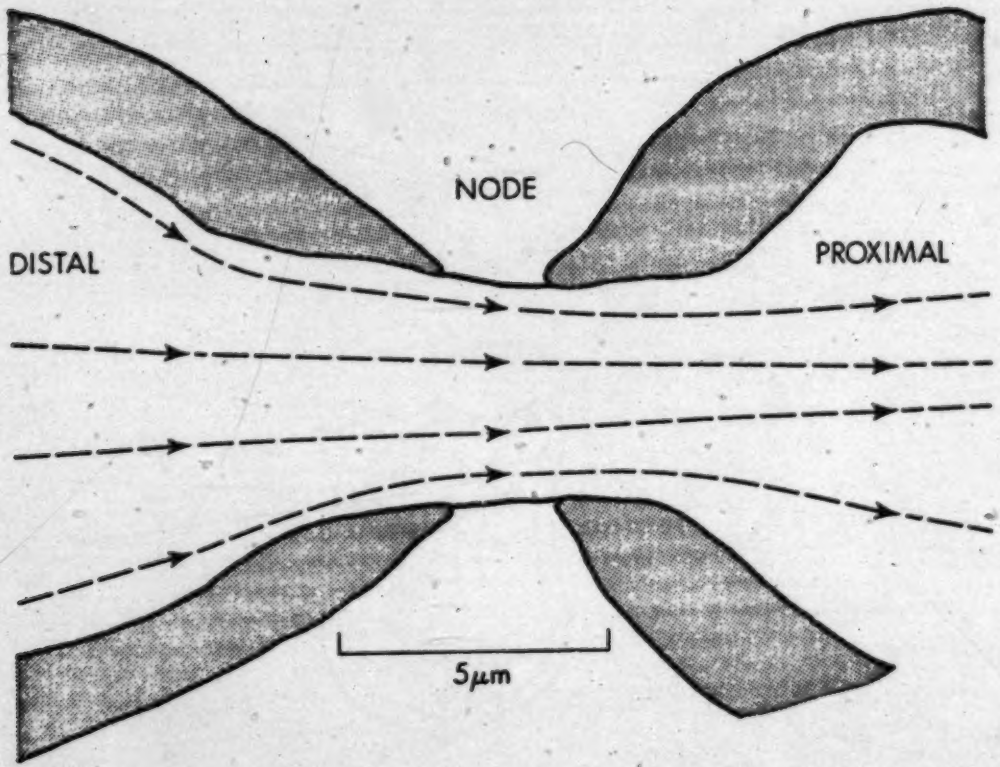


Figure 2.7. A node of Ranvier drawn from motion picture film of a sciatic nerve fiber. The myelin is shaded. The proximal, right hand, side of the node is the side closest to the spinal cord. Trajectories of particles crossing the node in the somatopetal direction are shown; dashed lines and arrows. The outer two trajectories represent the envelope of 50 particle trajectories.

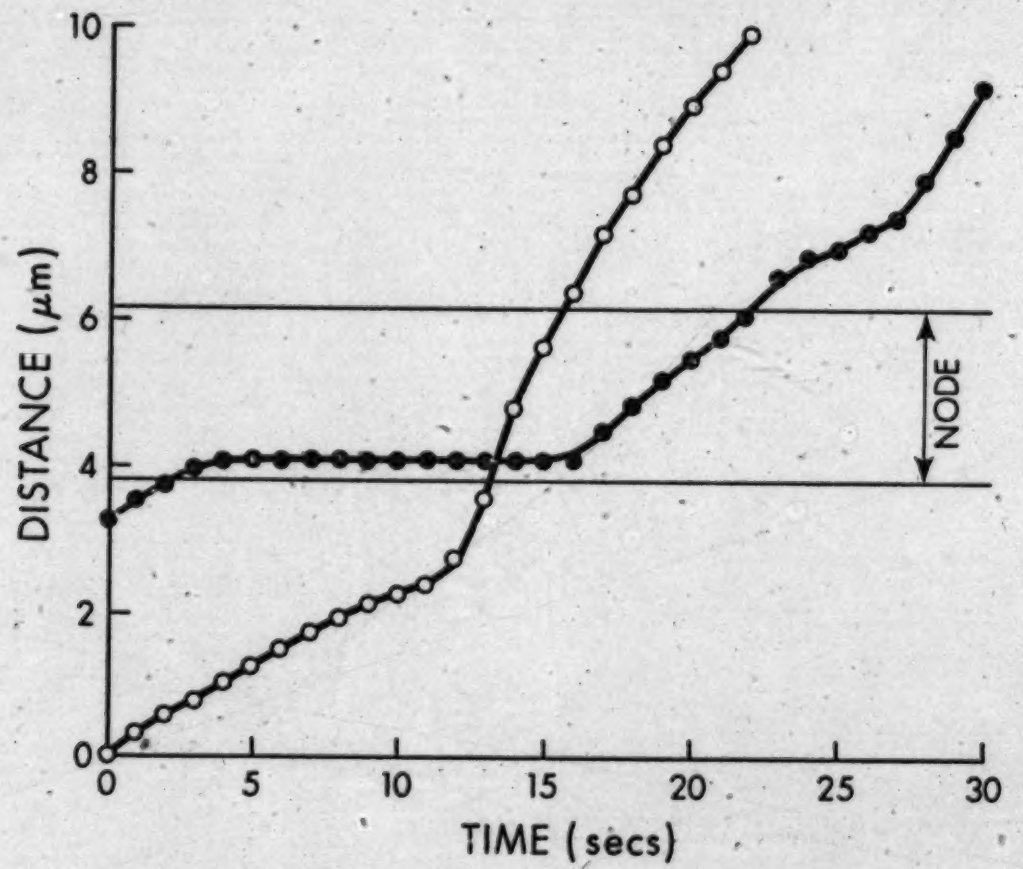


Figure 2.8. The motion of two particles crossing a node of Ranvier in the somatopetal direction. Position of the node indicated by horizontal lines. Filled circles indicate the motion of a particle which hesitated for about 10 sec on entering the node. Open circles indicate the motion of a particle which was not impeded at the node. In the latter case the beginning of a saltatory jump occurred just before the node. Note that the motions of the two particles do not appear to be related.

The hesitation of some organelles at the entrance to the nodal constriction caused a local decrease in the mean velocity of organelles. A group of 50 organelles crossing in the somatopetal direction took an average of 20.2 sec to traverse a 10 µm distance spanning the node as compared to an expected 10 sec for a similar distance in the internode. This slowing, however, would not appreciaby affect the transport rates over long stretches of axon.

MOTION OF THE ROD-SHAPED ORGANELLES

The majority of rod-shaped organelles remained stationary within the axoplasm. Extended periods of observation and motion picture films failed specifically to detect any movement at the slow axoplasmic transport rate (approximately 1 mm/24 hr or 40 µm/hr). Some rod-shaped organelles displayed a sudden axial shift in the axoplasm of up to 10 µm in either direction at velocities comparable to, or somewhat less than, those of most of the round organelles. These sudden shifts were only rarely followed by any further motion. More rarely yet, rods were seen moving with a continuous saltatory motion. Figure 2.9 shows the time course of displacement in the somatofugal direction of an organelle which was 0.3 µm wide and 3.5 µm long. Its course over 15 µm was taken from motion picture film. The dashed line of Figure 2.9 has a slope of 0.15 µm/sec and represents the velocity of the organelle over a 45 µm path in which the details of motion could not be entirely

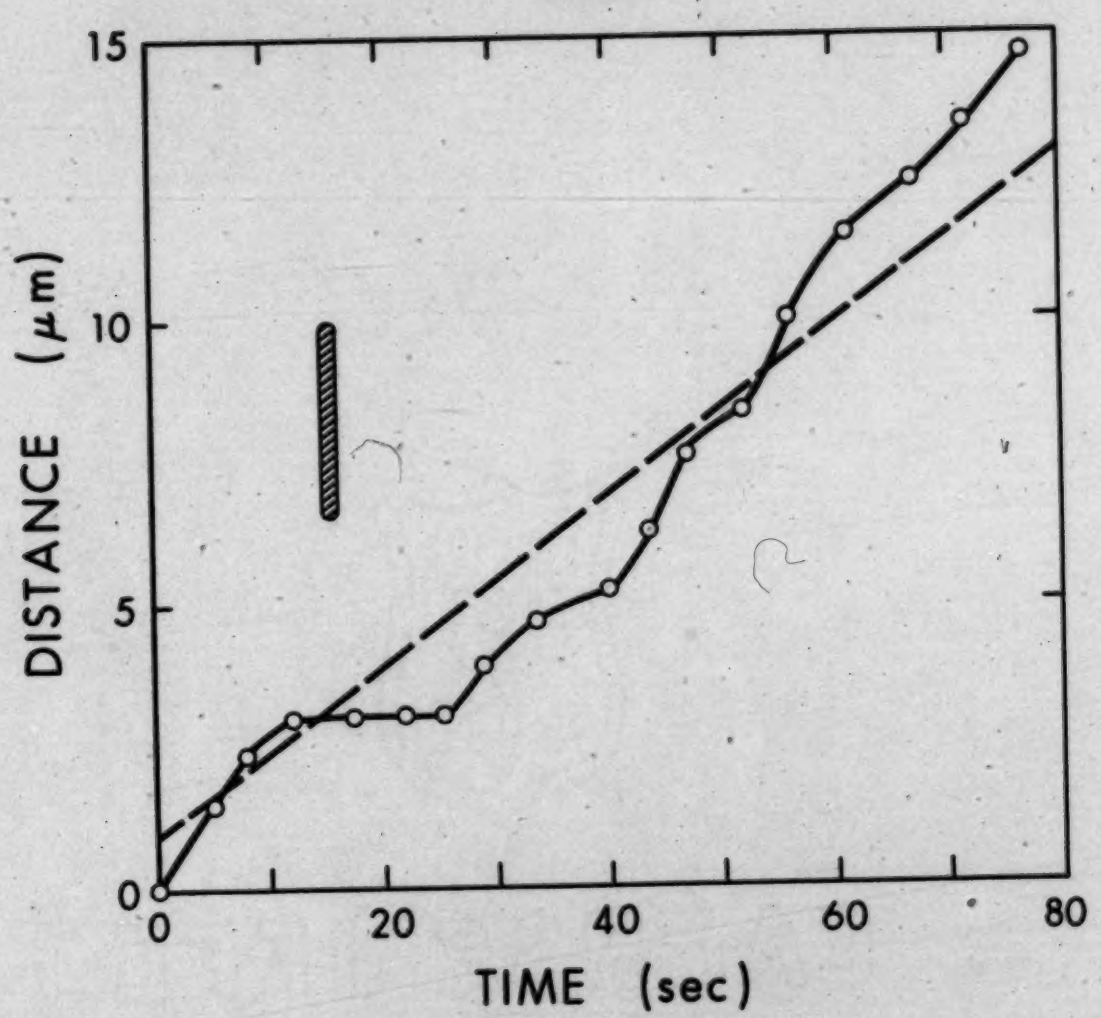


Figure 2.9. Motion of a rod-shaped organelle in the somatofugal direction. Inset is a diagram of the organelle which was 0.3 μm wide and 3.5 μm long (scale as on the ordinate of the graph). The organelle moved in a series of saltatory jumps at an average velocity (dashed line) of about 0.15 μm/sec.

observed since the organelle moved out of and back into the focal plane.

RELATIONSHIP BETWEEN THE MOTION OF ROUND ORGANELLES AND THE RODS

A very evident phenomenon was the tendency of the round organelles to run along the edge of stationary rod-shaped organelles. Particles travelling in both directions took paths alongside the same rod. No other preferred pathways were noted. A particularly interesting sequence was observed in a sciatic nerve fiber in which, at one location, the rod-shaped organelles were arranged in a waved, roughly sinusoidal, pattern. There was no evidence that this axon was damaged in any way. Round organelles entering this region in either direction followed axoplasmic pathways matching the arrangement of the rods. Figure 2.10a diagrams the beginning of a sequence in which a round organelle travelling somatopetally entered this region. Its curving pathway through the axoplasm included the edge of a rod-shaped organelle (Figure 2.10b). Within a second or two of the passage of the round organelle past the rod, the rod moved rapidly in the somatofugal direction. The pathway taken by the rod and the final position it assumed matched exactly the pathway previously taken by the round organelle (Figure 2.10c). These observations indicate that there is a relationship between the positions occupied by, and the movement pathways of, rod-shaped organelles and the pathways

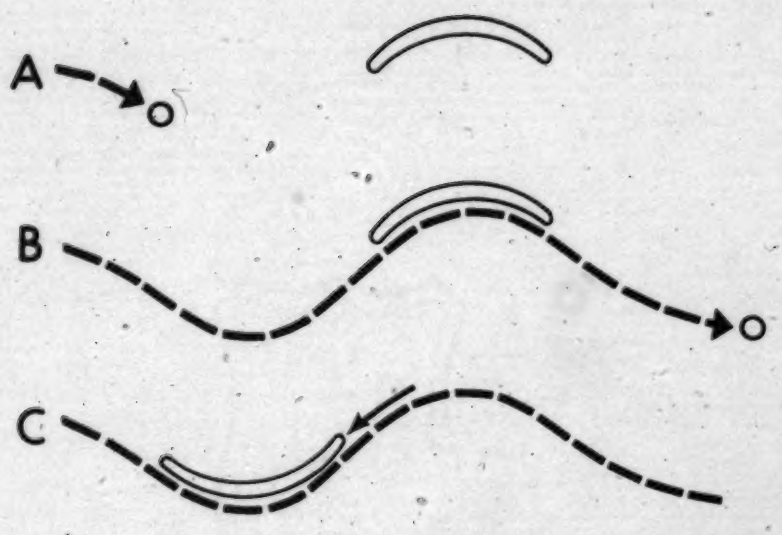


Figure 2.10. A diagram of a sequence in which a somatopetally travelling particle entered an area of folded axoplasm (A) and proceeded to travel along the edge of a rod-shaped organelle (B). A few seconds after the passage of the particle the rod moved along the path previously taken by the particle to a new stationary position as in C.

followed by round organelles. The simplest explanation would be that some structure, or axoplasmic track, associated with the mechanism of organelle movement runs along the edge of rod-shaped organelles.

DISCUSSION

PARTICLE TRANSPORT IN EXCISED SEGMENTS OF MATURE NERVES

Until very recently, it was still possible to regard sustained rapid movement of large organelles, especially in the retrograde direction, as a process which did not exist in normal mature axons. Weiss (1969b) advocated the view that this type of transport was a phenomenon peculiar to immature nerve cells, present only during a phase of active growth in fibers *in situ* or in tissue-cultured neurons. He conceived of axonal flow as a slow bulk movement of the entire "column of axonal substance", growing forth at a rate of one to several millimeters per day, exclusively in the distal direction. Although he did acknowledge the intra-axonal migration of large organelles such as mitochondria, he maintained that, except for active local excursions within a narrow range, they were carried along the axon passively and unidirectionally with the current of axonal flow (Weiss and Pillai, 1965).

This view of axonal transport afforded little recognition to the possibility of rapid bidirectional migration of large organelles in mature axons. Although

Weiss suggested the possible presence within the moving axonal column of separate channels for faster movement, and acknowledged the evidence suggesting some form of direct communication between the periphery of the nerve and its cell body, he claimed that his evidence positively ruled out the possibility of two-way substance transfer in mature nerve fibers (Weiss, 1969b). Reported observations of rapid particle movements in embryonic or regenerating nerve fibers were considered to be of dubious relevance, since "these immature or remobilized neurons are so different in state, consistency, and behavior from the mature nerve fiber that unsubstantiated transfer of conclusions from one to the other is quite unrealistic".

The direct microscopic detection of rapid bidirectional organelle movements in mature axons freshly isolated from adult vertebrates directly challenges such a limited concept of axonal transport. The results described in this chapter, and published in a separate paper (Cooper and Smith, 1974), are in general agreement with the observations reported in several earlier studies (Kirkpatrick, 1971; McMahan and Kuffler, 1971; Smith, 1971; Kirkpatrick, Bray and Palmer, 1972; Smith, 1972a,b; Kirkpatrick and Stern, 1973; Smith, 1973), and provide a more detailed account of axonal organelle transport under normal conditions than had previously appeared. Subsequent studies (Kirkpatrick and Stern, 1975; Smith and Koles, 1976; Forman, Padjen and Siggins, 1977a,b; Hammond, 1977; Hammond and Smith, 1977;

Heslop and Howes, 1977; Smith, 1980) have strongly supported the results reported here and have shown that a similar transport system is present in mature axons of a variety of vertebrates and invertebrates.

Since the preparations used in this and most other studies consisted of lengths of nerve isolated from the animal, it is not certain that the results represent the behavior of organelles in intact nerve cells. However, several lines of evidence do suggest that the condition of the nerve fibers used in these experiments was not greatly different from that of intact cells.

Most of the observations were made at distances of the order of 1000 fiber diameters from either cut end of the preparation. Fibers from the same animal are known to remain electrically intact for many hours at such distances from the cut ends (e.g. Hutchinson, Koles and Smith, 1970); presumably this reflects a fairly normal ionic milieu within the axoplasm. Close to the cut ends conditions must be abnormal but work such as that reported in Chapter 5 indicates that the abnormal zone is likely to lie within the terminal few millimeters of the preparation during the period when measurements were being made. It has been shown (Lubińska, 1956; Johnson, Smith and Lock, 1969) that axoplasm is generally not lost from the cut ends of the nerve, although axoplasm is transfered from the terminal few hundred micrometers of nerve to a myelin-covered ball at the

end of the nerve fiber. Both the rate of movement of the terminal axoplasm and the amount shifted are small, hence it did not seem likely that this effect would appreciably influence the measurements. Bulk transfer of axoplasm toward either cut end would, had it existed, have been detected in the motion pictures.

Saltatory particle movement in intact tissue-cultured neurons of Xenopus laevis takes place at maximal ("instantaneous") velocities ranging from 0.6 to 2.25 µm/sec (Berlinrood, McGee-Russell and Allen, 1972). Edström and Hanson (1973a) give 127 mm/day (1.5 µm/sec) for the fast somatofugal transport of radioactively labelled proteins in relatively intact frog neurons. More recently the velocity of rapidly, somatofugally, transported proteins has been measured in the sciatic nerve of Xenopus laevis giving a value of about 150 mm/day (Smith and Snyder, 1979). At least 60 mm/day is reported for the somatopetal transport of similar radioactive labels (Edström and Hanson, 1973b). Indirect measurements of the rates of movement of various enzymes in isolated segments of frog sciatic nerve (Partlow et al, 1972) give values varying between 11 and 99 mm/day (0.13 and 1.1 µm/sec), depending on the enzyme assayed and the direction of movement. The latter experiments were carried out over very long periods, up to 96 hours. The rates of particle movement obtained in this laboratory, and in the comparable preparations studied by Forman, Padjen and Siggins (1977a,b), are very close to those given in the work

cited above. In addition, Ochs and Ranish (1970) have produced evidence that fast axoplasmic transport in mammalian neurons is not dependent on the integrity of the whole nerve cell. Hammond (1977), in a direct comparison of particle motion in intact and sectioned amphibian axons, concluded that the movements were essentially similar. Lubińska (1975) has reviewed further evidence suggesting that the characteristics of axonal migration determined in interrupted nerves are probably valid for intact neurons.

DIRECTIONS AND VELOCITIES OF ORGANELLE MOVEMENTS

The observations by light microscopy of organelle movements in myelinated axons from chickens (Kirkpatrick, Bray and Palmer, 1972; Kirkpatrick and Palmer, 1972) and from humans (Kirkpatrick and Stern, 1973) were until recently the only other detailed reports available for direct comparison. In general the findings were very similar. Spherical particles of about 0.2-0.5 µm diameter moved both somatopetally and somatofugally. The velocity of these in chicken nerve was given as 1.24±0.48 µm/sec (mean ±s.d., n=46, temperature 31°C). Rod-shaped organelles were also present in the axoplasm and these were occasionally seen to move. There were, however, some notable specific differences between the results of Kirkpatrick et al and those reported here.

In both chicken and human nerves, the majority of round organelles were reported to move in the somatofugal

direction rather than in the somatopetal direction as observed in this laboratory, in mammals (Smith, 1972a) as well as in amphibians. This apparent contradiction persisted for some time until Kirkpatrick and Stern (1975) confirmed that they had been mistaken in their previous interpretation of the predominant direction of movement (see also Hammond and Smith, 1977). The recent work of Forman, Padjen and Siggins (1977a,b) has further verified the apparent predominance of the somatopetal movement.

Even so, the question of whether more organelles move in one direction than in the other has not yet been conclusively resolved. The fact that a given optical technique detects more particles moving in a certain direction does not necessarily mean that more material is being transported in that direction. The threshold for optical detection by either of the techniques used (Nomarski or darkfield optics) depends largely on the difference in the refractive indices between the object (an organelle) and its surrounds, and on geometrical factors such as the thickness of the object. The diameter threshold for detectability of the organelles in nerve has been estimated at about 0.2 um for darkfield optics (Smith, 1972a) and it is probably about the same for Nomarski optics used under the most favorable conditions. It follows that if one group of organelles, those moving somatofugally in these experiments, contains many organelles with a size less than the threshold for detection and if another group, those

moving somatopetally, contains organelles whose size is generally greater than the detection threshold, then more organelles will be observed moving somatopetally although the two groups may contain the same numbers of organelles. A similar argument could be used with respect to the density or refractive index of the organelles. The results are understandable if one views the process observed in the axon as part of a circulation of organelles which originate at the soma and increase in diameter or density as they mature and finally return toward the soma. The remaining possibility, which still does not rule out the influence of optical detectability, is that in Xenopus there are in fact more organelles moving somatopetally than somatofugally. Observations on the uptake and somatopetal transport of a variety of both radioactively labelled and non-labelled proteins by axons would lend support to this idea (see Chapter 1 and review by Schwab and Thoenen, 1977).

In addition to studying the transport of optically detectable organelles, Kirkpatrick, Bray and Palmer (1972) compared these movements with the migration of radioactively labelled proteins in chicken nerves. The fact that both forms of transport responded to certain chemical agents and incubation conditions was taken as an indication that the movement of the particles represented rapid axonal protein transport. A comparison of transport velocities was submitted as further support for this opinion. Comparing their average particle velocity of 106.8 mm/day at 31°C with

a cited rate of 312-360 mm/day at 37°C for radioactively labelled protein transport in chicken nerve, and allowing for an apparently arbitrary increase in velocity at the higher temperature, the authors judged the two rates to be similar, and interpreted this similarity as additional evidence for the identity of particle and protein transport. However, this judgment seems highly questionable. If the stated velocities had in fact characterized the same process at 31 and 37°C, calculation of the Q_{10} (Prosser, 1973) would have resulted in an exceptionally high value somewhere between 6.0 and 7.6. Several Q_{10} determinations have been reported in the literature, and range from 1.0 to 2.6 for radioactive protein transport (Lubińska, 1975) and from 2.5 to 3.5 for particle transport (Forman, Padjen and Siggins, 1977b).

Similarly with human nerve, Kirkpatrick and Stern (1973) stated that most recorded particle speeds were about 3 mm/hour. It is not clear how they arrived at this high a figure, since 13 of the 14 velocity measurements they reported were in the range from 1.2 to 2.8 mm/hour. Again a difference in temperature of 6°C (37-31°C) was proposed to account for anywhere from a 4- to 8-fold difference in velocity, depending on the specific assumptions made. Yet the claim was repeated that the observed organelle velocities fell "well within the range" of velocities determined for radioactive proteins, and that the optical detection method "accurately duplicates results from other

experimental methods".

While some relationship no doubt exists between the transport of large particles and protein transport, available evidence has always indicated that the relationship is neither simple nor complete. It is still premature to conclude that "the rapid movement of intra-axonal particles seen with Nomarski microscopy must represent rapid axoplasmic flow" (Kirkpatrick, Bray and Palmer, 1972). Even if legitimately similar velocities had been reported by Kirkpatrick and associates, the comparison would still have been questionable because they were unwittingly comparing somatopetal velocities for particle transport to somatofugal velocities for radioactive protein transport. The results reported in this chapter suggest that there may be a difference between mean particle velocities in the two directions, as is generally thought to be the case with protein transport (Heslop, 1975). However, the difficulties involved in reaching a definite conclusion are discussed in Chapter 3.

There was no indication in the present work of consistent organelle movement at a distinct slower velocity. Kirkpatrick, Bray and Palmer (1972), on the other hand, claimed to have detected a second class of particle movements corresponding to "slow" axonal flow. These slow movements were said to range from 50 to 100 μm/hour (1.2 to 2.4 mm/day), and were supposedly revealed in motion pictures

photographed at 12 frames per minute. Direct evidence of material being transported in this velocity range would be of considerable significance for the study of slow axonal flow. It might be surprising however to find such evidence in segments of axons surgically separated from their cell bodies, when slow transport is commonly thought to be dependent upon continuity between the axon and its functioning soma (Jeffrey and Austin, 1973). No other workers have reported slow particle movement despite the fact that some of the procedures employed should have been appropriate to reveal such movements. Even the original authors failed to make any further mention of slow movements in their subsequent reports. It may be pertinent that Heslop and Howes (1977), who specifically attempted to detect slow movement of particles in insect axons, were not successful using intact fibers, but did observe a slow flow of organelles in deteriorating preparations. It seems likely therefore that the original observations were artifactual, due either to utilizing axons in less than optimal condition, or resulting from the difficulty of obtaining acceptable photographic images under borderline optical conditions.

NATURE OF THE OPTICALLY DETECTABLE ORGANELLES

The paper by Matsumoto in 1920 is remarkable in that at that early date he identified, by means of vital staining, several classes of intra-axonal organelles in

tissue-cultured sympathetic neurons. He detected rod-shaped and granular mitochondria by staining with Janus black, and described a group of granules and some vacuoles that stained with neutral red. The mitochondria showed a "moderate degree" of movement while the granules which took up neutral red showed "considerable movement, especially in a longitudinal direction". In recent years it has been assumed by most workers that the optically detectable rod-shaped organelles in axons are mitochondria. This assumption is well supported by the long, axially oriented profiles of mitochondria that are seen by electron microscopy in thin sections of axons, and by the studies of mitochondrial shapes in some vertebrate nerves (Blume and Scharf, 1964). The nature of the round organelles is much more uncertain. From Matsumoto's work one can assume that in embryonic sympathetic nerves this group is composed of mitochondria (granules which stain with Janus black), and granules and vacoules which stain with neutral red. It has been suggested that a large proportion of the round organelles seen in motion in adult Xenopus axons are mitochondria (Smith, 1971, 1972a,c, 1973). Kirkpatrick and associates, in their papers on particle movement in chicken and human nerve (Kirkpatrick, Bray and Palmer, 1972; Kirkpatrick and Stern, 1973; Kirkpatrick and Stern, 1975), imply that the round organelles are something entirely different than mitochondria. In one paper (Kirkpatrick and Stern, 1973), the definitive claim is made that "The identity of the

particles seen by Nomarski microscopy was established by electron microscopy...spheres greater than 0.2 μ in diameter being moved through the axon...are identified as vesicles of smooth endoplasmic reticulum". No further explanation was given.

The complete identification of the moving organelles seen in axons under the light microscope will require more than a casual inspection of electron micrographs of comparable axonal sections. Two new approaches have recently been reported. Breuer et al (1975) have singled out a specific moving particle, and kept track of it from fixation under the light microscope throughout the stages involed in the preparation of the corresponding electron micrographs. Heslop and Howes (1977) have done the same with a specific region of axon. A second approach has been developed in this laboratory. Fresh axons are gently constricted or crushed in a solution which does not disrupt the transport process near the interruption. The moving particles accumulate on both sides of the obstruction in concentrations much higher than are normally available for analysis. An important advantage of this technique is that the electron micrographs prepared from these regions may also be used to study the transported organelles which are too small or too scarce to be detected by other methods. This aspect is considered further in Chapter 5. In all, it seems reasonable to conclude at this stage that the rod-shaped organelles are mitochondria, while the group of round organelles is heterogeneous, containing

unknown proportions of mitochondria, unidentified vesicles and other bodies.

MECHANISMS UNDERLYING THE MOVEMENT

The purpose of the present work is not to deal in depth with the complex problem of the mechanisms underlying the transport process. This subject has been discussed (Cooper and Smith, 1974) and there is considerable evidence supporting the involvement of microtubules (Hammond and Smith, 1977).

CHAPTER 3

VARIABILITY IN ORGANELLE TRANSPORT

INTRODUCTION

In Chapter 2 the directly observable characteristics of organelle movements along axons were described. The study of axonal transport by the optical method offers a potential means of investigating the underlying mechanisms at the level of the individual element of transported material. Preparations which are stable in vitro for at least 8 hours have allowed the initiation of pharmacological studies directed at an understanding of the transport mechanism (Hammond and Smith, 1977). This type of investigation, among others, is still hampered by a lack of sufficiently detailed descriptions of the normal phenomenon. One particularly awkward feature of retrograde particle transport in normal axons is an apparently large variation between axons in the average velocity at which the particles move, and in the amount of detectable moving material. While this variability has been noted in earlier reports (Smith and Koles, 1976; Hammond and Smith, 1977), no quantitative statistical studies have been carried out to estimate the extent to which the variability is significant, or to apportion the total variability between the various contributing sources.

This chapter presents the results of an investigation into differences between axons from Xenopus laevis in the

mean velocity of somatopetal particle transport and in the numbers of detectable particles which undergo transport. The results demonstrate a significant difference in these measures between axons within individual animals, but only insignificant differences between the animals themselves. The substantial differences in the average values from axon to axon were accompanied by proportional differences in the standard deviations, resulting in a stable and characteristic coefficient of variation. Variation in the quantity of particulate material transported by different axons was associated exclusively with differences in the spatial density of the particles, and was not correlated with either mean particle velocity or with axon size.

METHODS

Large diameter (13-22 μm) myelinated axons were isolated at the center of lengths of sciatic nerve taken from female Xenopus laevis, using the general procedures described in Chapter 2. To isolate up to four single axons from the same animal, the sciatic nerves from both legs were split longitudinally into two separate bundles. The nerves were dissected, stored and observed in a saline solution of composition (mM) NaCl, 112.0; KCl, 3.0; $MgCl_2$, 1.6; $CaCl_2$, 3.0; Na_2HPO_4, 2.6; NaH_2PO_4, 0.45; glucose, 5.0. The solution was gassed with oxygen before use.

Particle velocity was determined by dividing the distance travelled by a particle in the axial direction of

the nerve fiber by the time taken to travel that distance. A permanent record of times and distances was built up by activating the pen of a strip chart recorder as the particle image crossed successive lines on an ocular grid. All records in which the image crossed fewer than four grid lines before being lost from view were rejected. At the magnification used, the minimum distance over which velocities were measured was 11.7 μm. The correspondence between measurements obtained in this way and those obtained by a completely objective optical method has been documented (Smith and Koles, 1976). The correlation coefficient of 0.98 or greater commonly found between the variables of distance and time measured in this manner (Chapter 2) further supports the use of this direct method. The mean particle velocity for the individual axons was calculated by averaging the velocities of 50 consecutive particles in each axon.

To obtain a measure of the amount of visible particulate material moving within the axons, the times at which all detectable particles crossed a diameter of the axon were recorded on a strip chart recorder. The mean time interval between particles was calculated from the strip chart record. Axon diameters were measured with the ocular grid at the locations where the observations were made.

Following the observation of each axon, ambient temperature was measured in the air near the microscope

stage. A separate series of measurements (Smith, unpublished) showed that the temperature in the saline in the observation chamber was within 0.5°C of the ambient temperature, and that the saline temperature in the path of light from the dark field condenser remained within 0.2°C of that outside of the light path, as long as the heat filters were in place and the objective lens was coupled to the observation chamber with immersion oil.

The particle velocities and the quantities of detectable material transported were both examined using a nested analysis of variance (Sokal and Rohlf, 1969), random effects model. The specific version employed was the design CRH-3(12) given by Kirk (1968), with axons nested within animals.

RESULTS

PARTICLE VELOCITIES

The velocities of 50 particles were measured for each of 22 axons from seven animals at room temperatures that ranged from 22.0 to 23.1°C. The overall mean velocity of all 1100 somatopetally moving particles was 0.85 μm/sec (Figure 3.1), and the mean velocities for the individual axons ranged from 0.57 to 1.23 μm/sec. Figure 3.2 represents the complete set of results as the mean particle velocity for each axon surrounded by a 95% confidence interval for the mean. The figure clearly demonstrates that within individual

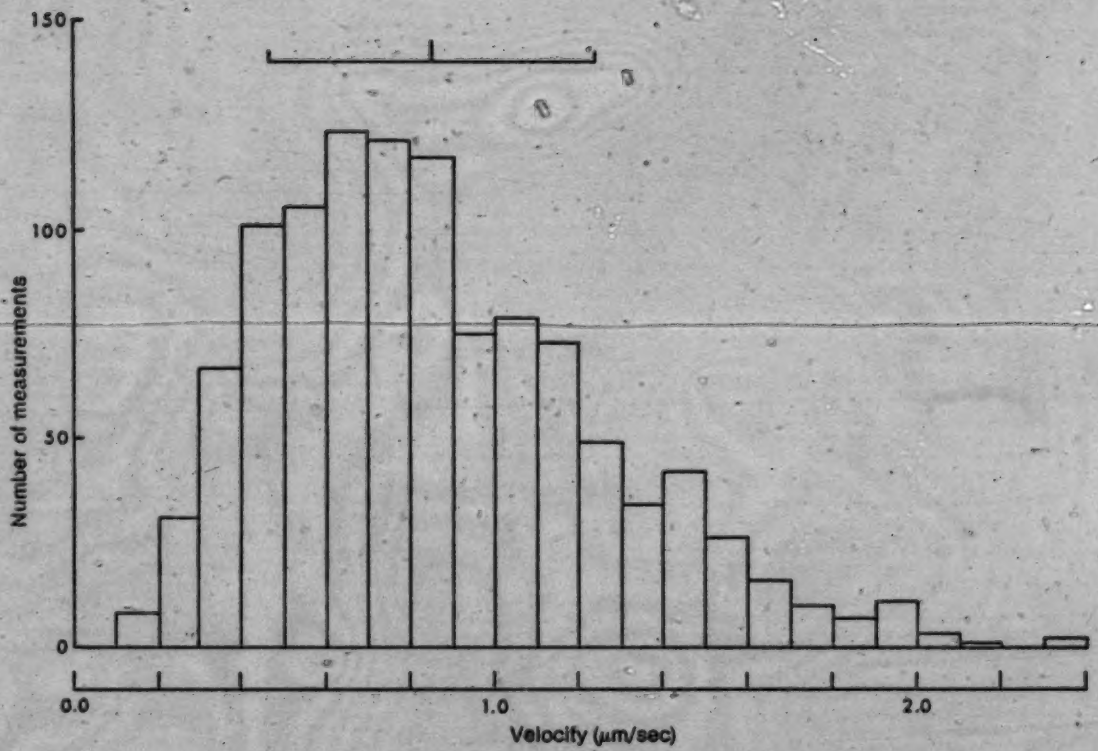


Figure 3.1. Distribution of average velocities of 1,100 somatopetally moving particles in sciatic nerve fibers of Xenopus laevis. Based on pooled samples of 50 particle velocities from each of 22 axons in 7 animals. Mean and standard deviation of distribution are indicated above histogram.

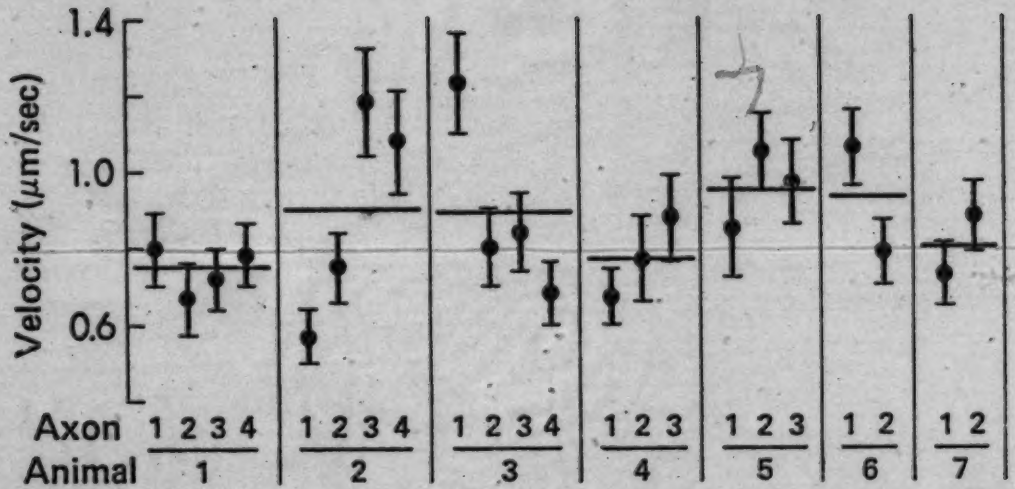


Figure 3.2. Mean velocities, with 95% confidence intervals for the means, for somatopetally moving particles in individual axons. Fifty particle velocities were measured in each of two, three or four sciatic nerve axons of seven Xenopus laevis. Horizontal lines represent the average of the mean velocities obtained from each animal.

animals a statistically significant difference in mean particle velocity could be found between some axons. However, only a limited variation appeared between the averaged values for each animal.

The results obtained from animals 1 to 3 of Figure 3.2, each of which contained samples from four axons, were subjected to an analysis of variance (Table 3.1). This analysis confirmed the impression given by Figure 3.2 in that the mean particle velocity between some axons differed significantly ($P<0.01$) while there was no significant difference ($P>0.05$) between animals. The proportion of the total variance attributable to each of the experimental components was calculated. Differences between the velocities of the individual particles accounted for 73% of the overall variance while 27% was associated with differences between individual axons; there was no detectable component between animals.

Illustrations of the extent to which the velocities of individual particles within single axons may vary have been given in other reports (Cooper and Smith, 1974; Forman, Padjen and Siggins, 1977a,b) and are essentially in agreement with the results given here. Figure 3.3, however, besides demonstrating the distribution of particle velocities in each of three axons for which the mean particle velocities were significantly different ($P<0.01$), also suggests that the variation in particle velocities

Table 3.1. Analysis of variance summary table for average velocities of somatopetally moving particles in sciatic nerve fibers of Xenopus laevis. Symbols: df, degrees of freedom; SS, sum of squares; MS, mean square; F, ratio of mean squares.

Source of variation	df	SS	MS	F	
between groups (between animals)	2	2.85	1.43	0.60	n.s.
between subgroups within groups (between axons within animals)	9	21.39	2.38	19.22	$p<0.01$
within subgroups (within axons)	588	72.73	0.12		
total	599	96.97			

Variance components	Proportion	Percent
within axons (between particles)	0.12	73%
between axons within animals	0.05	27%
between animals	0.00	0%

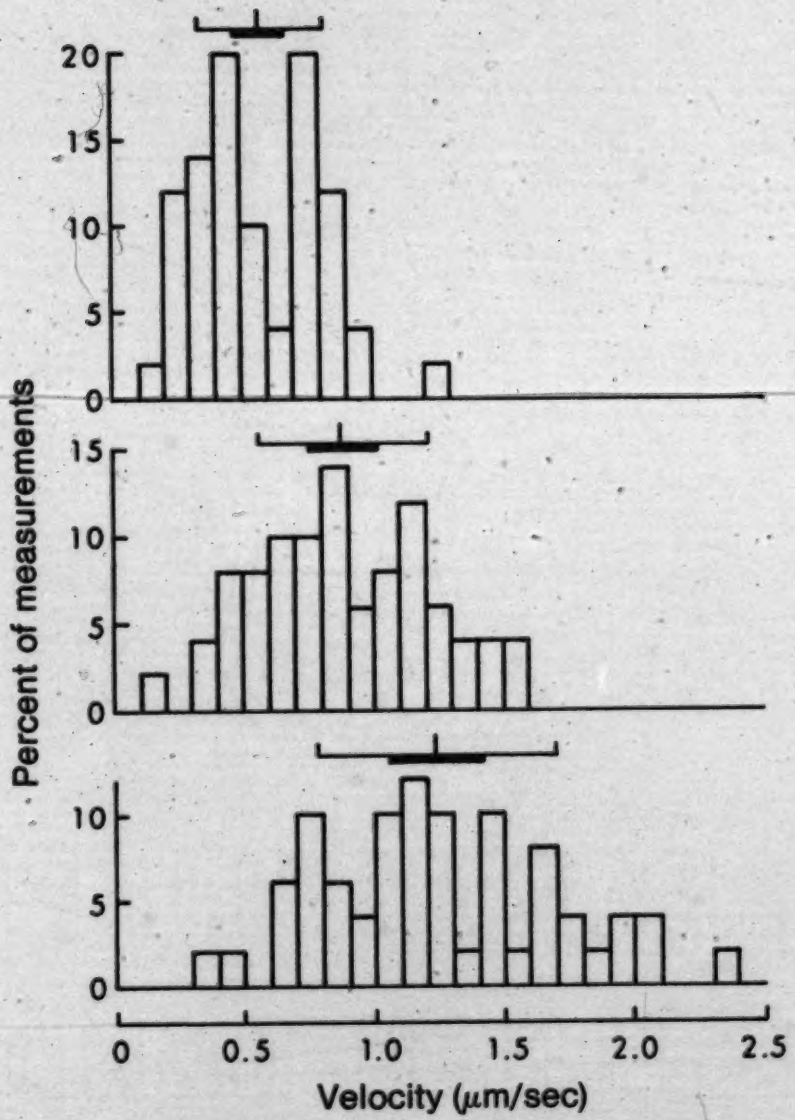


Figure 3.3. Distributions of particle velocities (n = 50) in each of three axons with significantly different mean velocities ($P<0.01$). Mean and standard deviation (fine lines), and 99% confidence intervals (heavy line), are indicated above each histogram. The increase in mean velocity in distributions A through C was accompanied by an increase in the variability between individual particle velocities (See also Figure 3.4).

increased with the mean particle velocity. Figure 3.4 shows a plot of the standard deviation (S) of each of the 22 samples of particle velocities against the corresponding mean velocity ($\bar{V}$). These data points were fitted by the least squares regression line $S = 0.34\bar{V} + 0.06$ with $r = 0.84$.

NUMBERS OF PARTICLES

Along with particle velocities, the second feature of the transport system which is most prominent under the light microscope is the rate at which the particles appear, or pass any particular cross section of the axon. This "traffic density" can be measured and quantified either as a count of particles per unit time, or as its reciprocal, the mean time elapsed between particles.

Time intervals between the arrival of consecutive particles at a cross section of the axon were recorded for a period of 5 minutes for each of the 22 axons which were used for the velocity measurements described above. The results were very similar to those obtained for particle velocities. Statistically significant differences again appeared between individual axons in several of the animals (Figure 3.5), while the variation between the averaged value for each animal remained small. The mean time intervals between particles, measured in individual axons, ranged from 13.4 to 2.8 seconds, corresponding to 4.5 to 21.3 particles per minute. The overall average time between particles was 7.9

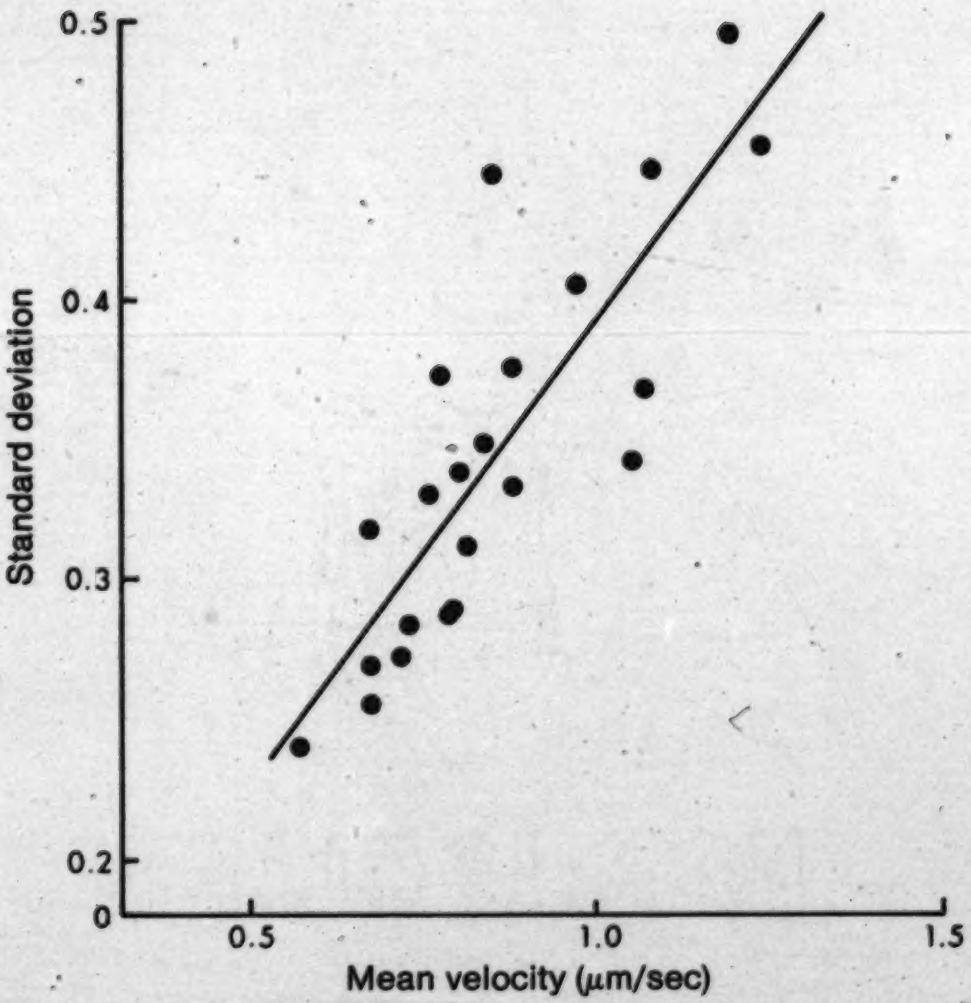


Figure 3.4. Standard deviation (S) against mean velocity ($\bar{V}$) in each of 22 axons at room temperature. Points fitted by least squares linear regression line $S = 0.34\bar{V} + 0.06$ with $r = 0.84$.

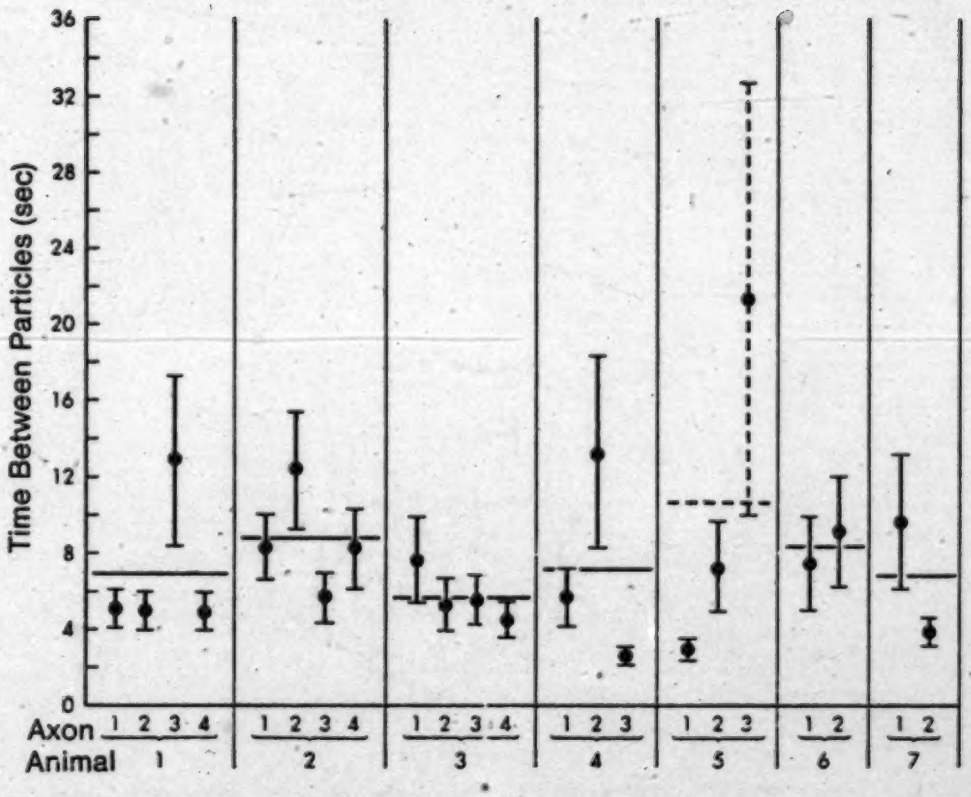


Figure 3.5. Mean time intervals between the arrival of particles at a given diameter of the axon, with 95% confidence intervals for the means, for somatopetally moving particles in individual axons. Horizontal lines represent the average of the mean times obtained from each animal. In one of the 22 experiments (broken lines), there was reason to suspect that the axon had suffered accidental damage.

seconds, representing 7.6 particles per minute.

The results of an analysis of variance based upon the numbers of particles per minute in the axons of animals 1 to 3 are summarized in Table 3.2. Within individual animals there was a significant ($P<0.01$) difference between some axons, while the differences between animals were not significant ($P>0.05$). The largest component of the total variance, 55%, was found within axons between the total numbers of particles counted in the respective 1 minute periods. A second major component, representing 39% of the total, was associated with the differences between axons within animals. Only 6% was found to be associated with the respective animals.

CORRELATIONS BETWEEN VARIABLES

The possibilities of correlations existing between certain combinations of mean particle velocities, mean number of particles per minute, axonal diameters and areas, and temperature were of interest for reasons described in the Discussion. All the calculated correlation coefficients were small however and none was statistically significant ($P>0.05$).

The results were also examined for the presence of time related effects which could have occurred in the serial isolation of up to four axons from one animal. There was no evidence that the duration of incubation of nerve bundles

Table 3.2. Analysis of variance summary table for numbers of somatopetally moving particles per minute in sciatic nerve fibers of Xenopus laevis. Symbols as for Table 3.1.

Source of variation	df	SS	MS	F	
between groups (between animals)	2	118.63	59.32	1.50	n.s.
between subgroups within groups (between axons within animals)	9	356.75	39.64	4.58	$p<0.01$
within subgroups (within axons)	48	415.60	8.66		
total	59	890.98			

Variance components	Proportion	Percent
within axons (between minutes)	8.66	55%
between axons within animals	6.20	39%
between animals	0.98	6%

(up to 12 hours) had any effect on the results.

DISCUSSION

SOURCES OF VARIABILITY

A complete account of axonal organelle transport involves descriptions and comparisons at several levels, each of which is accompanied by its own characteristic degree of variability. Variation is present in the movement of each individual particle from one moment to the next, between the different particles in any given axon, between the different axons in a given nerve or animal, between different animals of the same species in similar or different environmental and physiological conditions, and between different species or classes of organisms. The magnitude of these variations can differ greatly, but their comparative importance has not previously been studied in detail. Objective statistical estimates have been employed in the present analysis to separate sources of variation into those which are inconsequential and into those with important consequences for the design and interpretation of experiments on particle transport in axons.

VARIATION ASSOCIATED WITH INDIVIDUAL PARTICLES

It is immediately obvious under the light microscope that the velocity of individual particles varies considerably from moment to moment, but these movement patterns are too complex to be quantified directly. They

must be recorded in a permanent form and, due to the detail involved, are more appropriately analyzed by semi-automatic and computer based methods (e.g. Forman, Padjen and Siggins, 1977a). This type of strategy constitutes an approach beyond the scope of the present work.

The most basic directly measurable characteristic of movement is the average velocity of a particular particle over a representative distance. Analysis of variance showed that the differences between the average velocities of individual particles within axons was the major source of variation in the categories sampled, accounting for 73% of the total variation. This source might be expected to be large, since the velocity distributions typically covered an approximately 10-fold range, roughly from 0.2 μm/sec to 2.0 μm/sec. A further consequence of the broad distributions is the high values of the coefficients of variation (mean c.v. = 0.4, s.e.m. = 0.01, n = 22). In practical terms this means that the standard deviation accompanying any estimate of the mean velocity will regularly be almost one half of the value of the mean, making it necessary to measure the velocities of a sample of anywhere from 20 to 50 or more different particles in order to establish a reasonably reliable estimate of mean velocity for any given axon. Because of this characteristic, the higher the mean velocity of a sample of particles, the more uncertainty there is involved in the estimation of the mean velocity of the population.

The analysis of variance based upon the numbers of somatopetally moving particles per minute indicated that the major source of variability in this property, 55% of the total, was found within the individual axons. It is evident from these results that the transport process is characterized by a substantial variability within individual axons in both mean particle velocity and in amount of visible particulate material transported per unit time. The importance of this source of variability has just recently begun to be recognized, in both mature axons (Smith and Koles, 1976; Forman, Padjen and Siggins, 1977a,b) and in embryonic cultured axons (Breuer et al, 1975).

VARIATION BETWEEN AXONS

While the variability inherent in the transport system within individual axons has been recognized in the literature, the differences existing between individual axons has not been generally appreciated. On the contrary, the velocity distribution, average velocity and other statistical parameters estimated by combining measurements from several particles in a given single axon have been considered to be the same as, or similar to those in different axons studied at the same temperature (Forman, Padjen and Siggins, 1975a,b, 1977a). The subjectively less conspicuous nature of the inter-axonal variability, combined with the difficulties which have to be overcome in order to obtain statistically reliable data, are largely responsible

for the failure to recognize this source of variation. Figures 3.2 and 3.5 illustrate how easily similar mean velocities or particle counts could arise by chance in a random sample of a small number of axons.

To date, only Smith and Koles (1976) have acknowledged the appreciable magnitude of the inter-axonal variation in mean particle velocities. This result has been confirmed and quantified by the present statistical analysis, which employed a much more extensive and representative sampling procedure than has previously been used, to provide a quantitative estimate of the variation between different axons. This investigation has demonstrated that statistically significant differences in both mean particle velocities and in numbers of particles transported per unit time exist between different individual axons, irrespective of whether the axons belong to the same or different animals. These differences, which ranged up to 54% and 79% respectively, were responsible for 27% and 39% of the total variance in the samples.

It is clear from this analysis that the differences between individual axons must be taken into consideration in both the design and the interpretation of experiments on axonal particle transport. It has been common practice to combine a relatively small number of velocity measurements from each of several different axons in order to build up a large collective distribution, which was usually used for

the calculation of mean velocity and other statistics. It is evident now that such distributions may be expected to combine measurements from heterogeneous rather than homogeneous populations of particles, and are therefore appropriate only in situations where a generalization is required to represent the overall behavior of particles in a group of individual fibers, as in a whole nerve.

It is undesirable to use the composite distributions obtained in this manner as a basis for comparison of particle velocities or counts under different experimental conditions. The effects of the experimental variables are likely to be confused with inherent differences between the axons involved. Ideally, experimental effects should be studied within individual axons. However, the techninal difficulties involved in using individual axons as their own controls may be prohibitive or require further development (see Chapter 4). Where this approach is not feasible, it is important to minimize the sampling error involved by including as many axons as possible in each category. Factors considered under the following headings are also relevant to the effort to reduce sampling error.

The constant magnitude of the coefficient of variation in different axons with a wide range of mean velocities has been documented by Forman, Padjen and Siggins (1977b) and by Smith and Cooper (1979) by exposing the axons to different temperatures to raise or lower the velocities of the

particles. However it has been demonstrated by this series of experiments that the same relationship holds at a single temperature, when the differences in particle velocities are solely the result of the natural biological variation of the transport system.

The findings suggest that the individual particle velocities increase proportionately with the mean velocity, and this implies that some general factor operates proportionately on all the particles to determine their velocities. As velocity increases, this could be either a general increase in the driving force or a general decrease in the resistance to movement. Whatever specific factors are involved, they must be of such a nature that their final effect acts as a multiplier of each particle's previous velocity.

VARIATION BETWEEN ANIMALS

Before this project was undertaken, the uncertainty surrounding the degree of variation between different animals was similar to that concerning the variability between different axons; it was not great enough to be obvious, but neither was there any evidence that it was not sufficient to appreciably influence experimental results.

In the analysis of variance based upon somatopetal particle velocities (Table 3.1) it was found that no detectable variability was attributable to the different

animals in the sample. In the analysis based upon numbers of particles per minute (Table 3.2), only 6% of the total variance was associated with the differences between the individual animals. Neither of these components was statistically significant. Therefore, in experiments involving comparisons across both axons and animals, in order to achieve the best estimates of population parameters, it is important to work with samples consisting of a large number of particles per axon, and a relatively large number of axons, but not necessarily a large number of animals.

CORRELATIONS BETWEEN VARIABLES

The correlation coefficients between number of particles per minute and both axonal diameter and approximate cross sectional area were small and not statistically significant. There was apparently no more visible particulate material transported by the large axons than by the smaller ones, in the range from 13.3 to 21.9 μm outside diameter. Calculations based on the inside diameters and areas gave the same results. Although this conclusion was further confirmed by the study reported in the following chapter, it is extremely doubtful that this lack of correlation extends down to the smallest fibers.

There were also no significant correlations between the mean particle velocities and either axonal diameters or cross sectional areas. This is in agreement with the report

that the rate of ^3H leucine transport was independent of axonal diameter from about 3 to 23 μm (Ochs, 1972a). Because of the complete absence of any evidence for a relationship between the sizes of the axons and either particle velocities or numbers per minute, no correction factor was applied for axon size.

The relationship between particle velocities, the number of particles passing by per unit time, and the amount of material transported per unit time is described by the expression:

$$Q_t = D\ A\ V$$

where Q_t = quantity of material transported per unit time

D = density = quantity of material per unit volume

A = cross sectional area

V = velocity

With the present techniques the particle count per unit time is a direct measurement of Q_t, the velocity V is also measured directly, and the area A may be approximately calculated from the measured diameter of the axon. On the assumption that internal axonal structure, including particle density, is fairly consistent from axon to axon, it would be expected that differences in Q_t would depend upon differences in A or V. However, since both A and V varied independently of Q_t, it is concluded that the spatial density of the particles, which is difficult to measure directly, must differ considerably between different axons, and that this variable is the principal determinant of the

amount of visible particulate material transported along different axons.

The possibility that the results could have been due to a steep temperature dependence over a narrow range of room temperatures was not supported by the data. An extensive examination of the relationship between temperature and particle velocity (Smith and Cooper, 1979) further supported the conclusion that a difference of 1.1°C was not sufficient to account for the variation observed.

CHAPTER 4

ORGANELLE TRANSPORT IN LOCALLY CRUSHED AXONS AT TIMES OF ONE DAY AND LONGER AFTER THE CRUSH

INTRODUCTION

There have been no reports to date on the transport of optically detectable organelles in regenerating axons. The existing literature on axonal transport in regenerating nerve has been reviewed in Chapter 1 and it was noted that the presently available information is such that the nature of any changes occurring in axonal transport during regeneration is unclear. However, it was suggested there that the velocity of rapid transport might remain unchanged while the cellular requirements of regeneration could be met through alterations in the kinds and amounts of materials in transit.

Because of the lack of information on axonal transport in general, and on organelle transport in particular, during regeneration, an investigation was made of the transport of optically detectable particles in regenerating axons. Additional reasons for this study were the probable central importance of axonal transport to nerve regeneration (as discussed in Chapter 1), and the prospect of gaining insights into the transport of large organelles by examining the process in cells which were definitely abnormal. The results of the investigation support the suggestion derived

from the literature; no change in organelle velocity was detected but other aspects of organelle transport were altered.

METHODS

Adult *Xenopus laevis* were anesthetized with 2% urethane. The sciatic nerve together with its tibial and peroneal branches was exposed and was crushed with fine forceps at the junction of the tibial and peroneal branches. Care was taken not to damage the blood vessels in the region of the crush. The length of the crushed region was about 0.5 mm. The animals were allowed to recover from the anesthetic in a shallow pool of water. On recovery they were returned to tanks of deep water at room temperature where they swam freely.

Observations of particle transport were made on the nerves in two series of experiments. The first set of experiments used 17 animals. The previously crushed nerves were prepared for observation or filming of single axons, as described in Chapter 2, at locations 1 cm from the crush. Axons proximal to the crush were observed at 1, 2, 4, 8, 16, 32, 64, 96 and 128 days following the crush. Axons distal to the crush were examined 1, 2, 3 and 4 days after crushing. Motion picture records for detailed examination were made of one axon proximal to the crush at each time interval. Particle transport was observed at comparable locations in a number of other axons, in the process of selecting

preparations with satisfactory optical conditions for filming.

The motion picture film was used to obtain particle velocities, the numbers of particles crossing a diameter of the axon per unit time (average of three independent counts made at left side, center and right side of field), the numbers of rod-shaped organelles in motion during the 30 minute film sequence, and the diameters of the images of the particles. Image diameters were measured with a scale matching the spacing on the projected image of a microscopic calibration grid. In the case of particle images which were slightly elongated, the lengths of the major and minor axes were averaged. A set of observations of image diameters of latex beads with diameters ranging from 0.2 to 0.8 μm indicated that this method overestimates the particle diameter and that the image diameter is influenced by the suspension medium.

The survey suggested that a larger sample of axons should be observed. In the second set of experiments a group of axons was teased from each crushed nerve and the preparation was mounted in a pool of physiological saline confined by a Vaseline ring on a microscope slide. A drop of silicone oil (Dow Corning 710) was applied over the teased fibers and a cover slip was put over the entire preparation. The surface tension at the oil-water interface tended to spread the teased fibers into a monolayered mat, and also

had the effect of producing very good optics for viewing intra-axonal particles through the myelin sheath. A number of axons from each preparation could be observed. Using this method the numbers of particles passing a diameter of the axon per unit time, within a focal plane, were measured 1 cm from the crush at postoperative times of 1, 2, 4, 8, 16, 34, 64, 69 and 100 days.

The experimental results were compared to results obtained from normal animals at the same season of the year or with those obtained from the contralateral unoperated limb.

The progress of the regenerating nerves was checked functionally and histologically. Functional tests consisted of observation of the swimming behavior of the animal, its response to pinching the foot, and the observation of muscle contraction (or lack of contraction) on stimulating the proximal end of the sciatic nerve when the animal was sacrificed. Nerves from a separate series of animals with identical lesions, and recovery times up to 24 days, were examined in teased preparations which had been stained with a silver stain (Winkelmann and Schmit, 1957). Regenerating axons were also examined by electron microscopy in an attempt to identify organelles which were larger than those in normal axons. Regenerating and normal nerves were desheathed and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at room temperature for 2 hours. The

preparations were washed in buffer, treated with 1% osmium tetroxide in buffer, dehydrated in a series of alcohols and taken via propylene oxide to Epon. Sections were cut by Mrs. Sita Prasad with a diamond knife, stained with uranyl acetate and lead citrate, and examined with a Zeiss 9A electron microscope.

RESULTS

ASSESSMENT OF REGENERATION

Microscopic examination of axons teased from the silver stained nerves at 1-24 days after crushing indicated that the axoplasm initially receded about 0.4-0.5 mm proximally from the edge of the applied crush. Individual axons varied considerably in the rapidity of their response to injury, but after only 24 hours, approximately one third of the fibers exhibited clearly developed growth cones. Since the new sprouts originated at different times and at different distances from the crush zone, and grew at somewhat different rates, emphasis was placed for descriptive purposes upon the fibers showing the most advanced development. The maximum length of the 1 day sprouts was 0.35 mm, so that those whose origins had receded least had just reached the proximal edge of the crush zone.

By 2 days, half or more of the axons examined had produced sprouts. Their average length was 1.2 mm, so that some of the more vigorous fibers had grown completely across

the half millimeter crush zone and had entered the distal stump. In the axons which had regenerated for longer periods, the amount of stained material present made individual details more difficult to distinguish. It was evident, however, that a large proportion of the fibers continued to grow into the distal portion of the nerve, at a rate in the vicinity of 1 mm/day.

Functional indications, including spontaneous use of the operated limbs by the recovering animals, reflex responses to sensory stimuli, and muscular twitching on stimulation of the proximal end of the nerve during dissection, were in agreement with the morphological observations. All indicated a complete loss of transmission after crushing, and reinnervation with functional motor and sensory recovery by about 2 months, consistent with the estimated growth rate of around 1 mm/day.

GENERAL OBSERVATIONS ON PARTICLE TRANSPORT

Possibly the most striking finding was that in axons proximal to the crush, bidirectional particle transport was present at all time periods studied (1-128 days). Distal to the crush bidirectional particle transport could be detected for about 3 days.

Despite the large variability, the numbers of somatopetally moving particles proximal to the crush was substantially depressed within the first few days after the

operation. The amount of particle traffic in both directions recovered to at least normal levels by 1 week after crushing the axon. At all times following the crush the survey experiments showed an elevated number of rod-shaped organelles in motion in each direction. During regeneration a class of large, rounded organelles appeared in the proximal portions of the axons; these always moved in the somatopetal direction.

The mean velocities of somatopetally moving particles in normal and regenerating axons are compared in Figure 4.1A and B. Similarly, Figure 4.1C and D compares somatofugal particle velocities in normal axons and regenerating axons. Detailed examination of particle movement revealed no difference between the motion of particles in normal axons and their motion in regenerating nerve.

The changes in these characteristics of organelle transport following a crush lesion to the nerve will be treated in more detail below.

ORGANELLE TRANSPORT PROXIMAL TO THE LESION

Numbers of Particles Transported

The numbers of particles which crossed a diameter of an axon within one focal plane are shown in Figure 4.2A (somatopetally moving particles) and 4.2B (somatofugally moving particles) over the period from 1 to 100 days after

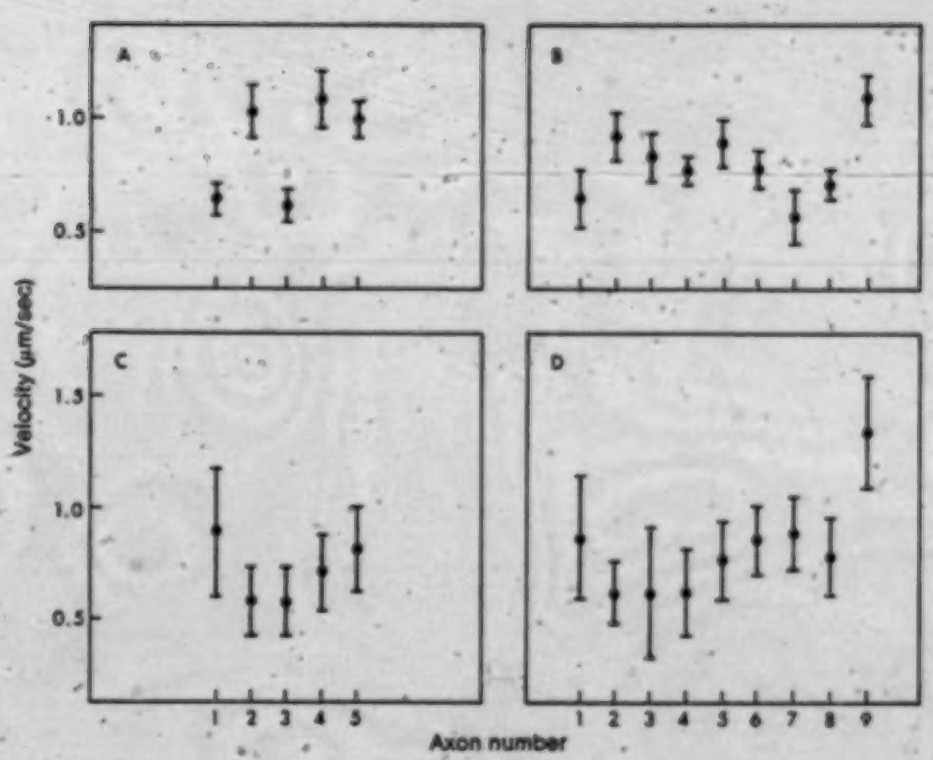


Figure 4.1. Comparison of mean somatopetal particle velocities in normal (A) and regenerating (B) axons, and of mean somatofugal particle velocities in normal (C) and regenerating (D) axons. Ninety-five percent confidence intervals for the means are also indicated. The nine regenerating axons were observed at successive periods from 1 to 128 days after crushing, in the proximal portion of the axons. The normal axons were taken from five different animals.

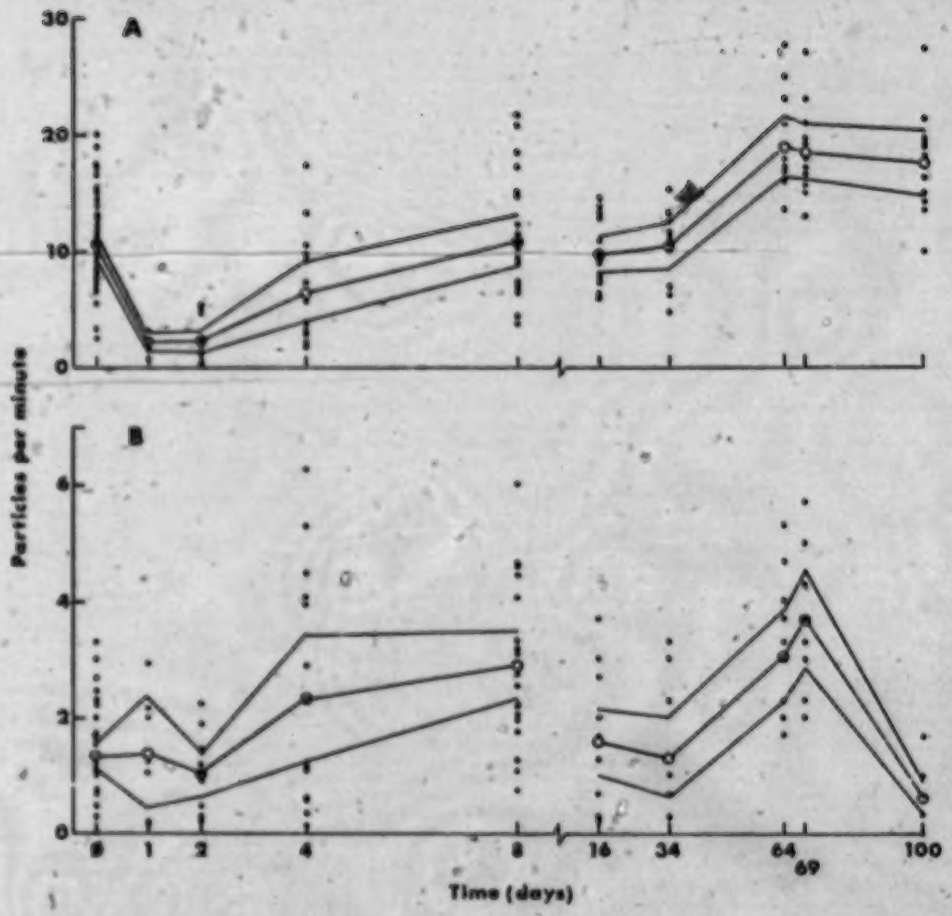


Figure 4.2. Numbers of somatopetally (A) and somatofugally (B) moving particles which crossed a diameter of the axon per minute in normal axons (Ø) and in axons proximal to crushes applied at intervals from 1 to 100 days previously. At these time periods, solid circles represent the average number of particles per minute during the observation period in individual axons, and open circles (on center lines) represent the mean for all axons observed at each time interval. The normal values (solid circles) are the averages in each of at least five axons from the sciatic nerve of the unoperated leg at each of the nine time intervals above; all of these samples were combined to determine the overall mean for the control axons (open circles). The upper and lower lines connect 95% confidence limits for the means. (See also Figure 4.3).

the nerve had been crushed. Values obtained from uncrushed contralateral axons at 1-100 days postoperatively were pooled and are plotted at zero on the abscissae as normal values. Somatopetal particle traffic, while present, was significantly depressed at 1 and 2 days ($P<0.001$) and at 4 days ($P<0.05$), compared to the control values. No significant departures from normal levels were observed from 8 to 34 days, but a significant increase in numbers occurred at 64, 69 and 100 days ($P<0.01$). The survey out to 128 days indicated that somatopetal particle traffic continued at or above a normal level. Somatofugal particle traffic was elevated ($P<0.05$) at 8, 64 and 69 days after the crush, and reduced ($P<0.05$) at 100 days.

For somatopetally moving particles the mean numbers per unit time, travelling in axons on the operated (OP) and unoperated (CTRL) sides, were significantly related ($P<0.025$). The data points were fitted by the least squares regression line CTRL = 0.31(OP) + 6.81 with $r = 0.70$. This suggested that retrograde transport is sensitive to systemic factors as well as to conditions within the damaged neurons. This finding also implies that to extract information which is of significance for regeneration per se experimental findings in the regenerating axons should be expressed in relation to comparable values from the unoperated side. Figure 4.3 shows the mean numbers of somatopetally (A) and somatofugally (B) moving particles as a percentage of the values in the corresponding control axons.

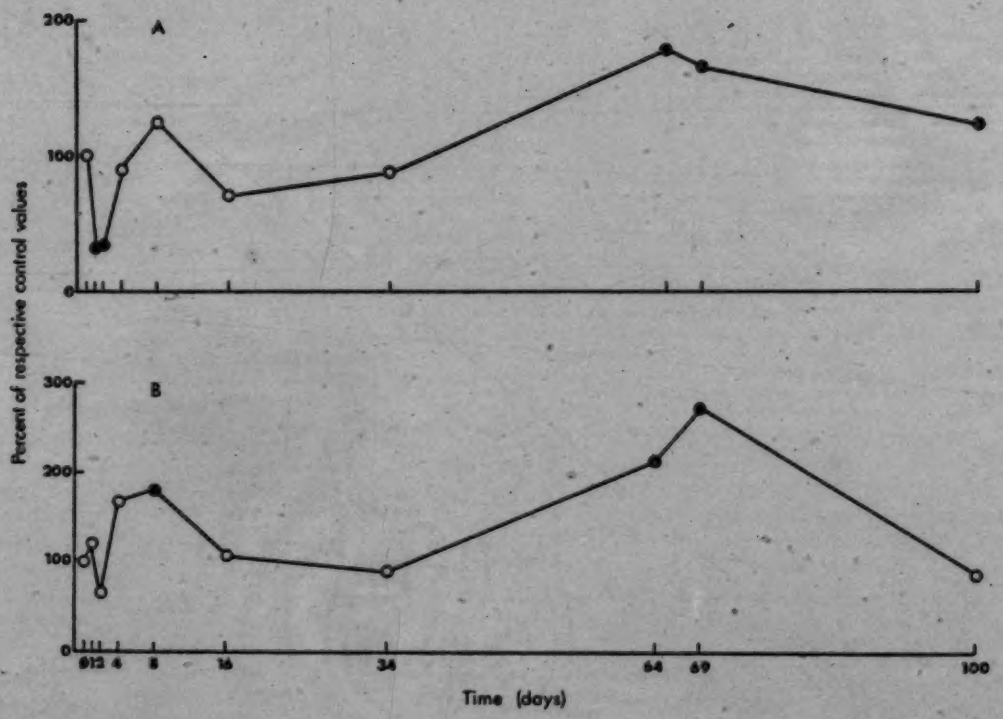


Figure 4.3. Changes following crush injury in mean numbers of somatopetally (A) and somatofugally (B) travelling particles in the proximal portion of axons at successive time periods, expressed in terms of the mean numbers on the unoperated side of the same animal. Solid circles indicate statistically significant ($P<0.05$) departures from mean numbers in samples of axons from normal animals (see text for discussion).

Numbers of Rod-shaped Organelles in Motion

As noted in Chapter 2, rod-shaped organelles were usually stationary in normal axons but some were occasionally seen to be in continuous motion. Since this kind of movement appeared to be more common in regenerating axons, a comparison was made between 30 minute filmed records from five normal nerves and the pooled results from similar records of nine nerves at regeneration times between 1 and 128 days. Normal axons showed 6.9±3.5 (mean±s.d.) rod-shaped organelles/hr moving in the somatopetal direction and 9.0±5.5 organelles/hr moving in the somatofugal direction. These values were not significantly different. In the total set of regenerating axons 17.7±5.6 organelles/hr moved in the somatopetal direction while 36.4±17.1 organelles/hr moved in the somatofugal direction. These values are different from the corresponding normal values and from each other ($P<0.01$).

Thus the conclusion that can be drawn is that following a crush lesion the numbers of moving rod-shaped organelles in the axon proximal to the crush is increased, and the number of such organelles moving towards the regenerating portion of the nerve is greater than the number moving away from it. The filmed records gave no evidence that this change in transport was confined to any particular time period after the lesion.

The Size of Particulate Images

The diameters of the projected images of 479 somatopetally moving particles were measured in five normal axons. The distributions were approximately normal with an overall mean (±s.d.) of 0.41±0.12 μm. No image diameter greater than 0.7 μm was observed. A corresponding set of 700 measurements was taken from nerves which had been crushed from 1 to 128 days previously; the mean diameter of the particle images was 0.46±0.15 μm. The difference between the two means, although small, was statistically significant ($P<0.001$). A factor contributing to the difference between the two sets of mean diameters was the appearance, in regenerating axons, of a set of particles with image diameters up to 1.1 μm. Many of these had a distinct microscopic appearance characterized by a highly refractile circumference with a dark center. This different appearance may have been an optical artifact rather than an indication of a qualitative difference between these and other, smaller, organelles. The velocities and the general characteristics of the motion of these organelles were consistent with those described in Chapter 2 for smaller somatopetally moving particles. No very large organelle was ever seen to move somatofugally.

Figure 4.4 shows the percentage of each sample of image diameters which was greater than 0.7 μm as a function of the time following the crush. Large organelles were present at

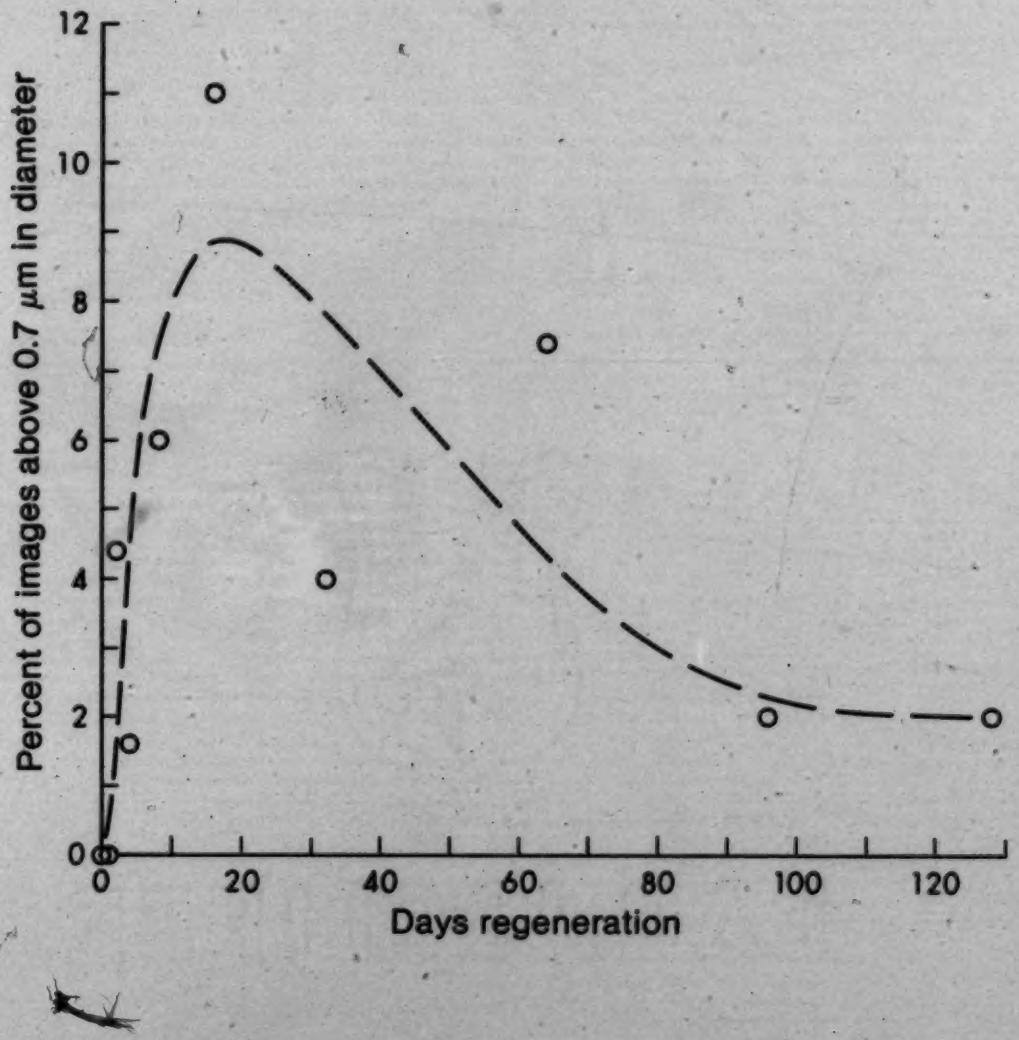


Figure 4.4. Proportion of somatopetally moving particles at each regeneration period with image diameters beyond the maximum size of 0.7 µm observed in a large sample of particles (n = 479) in normal axons. Sample size in each of the regenerating axons is 50 particles. Dashed line represents hypothetical relationship between image diameter and regeneration time.

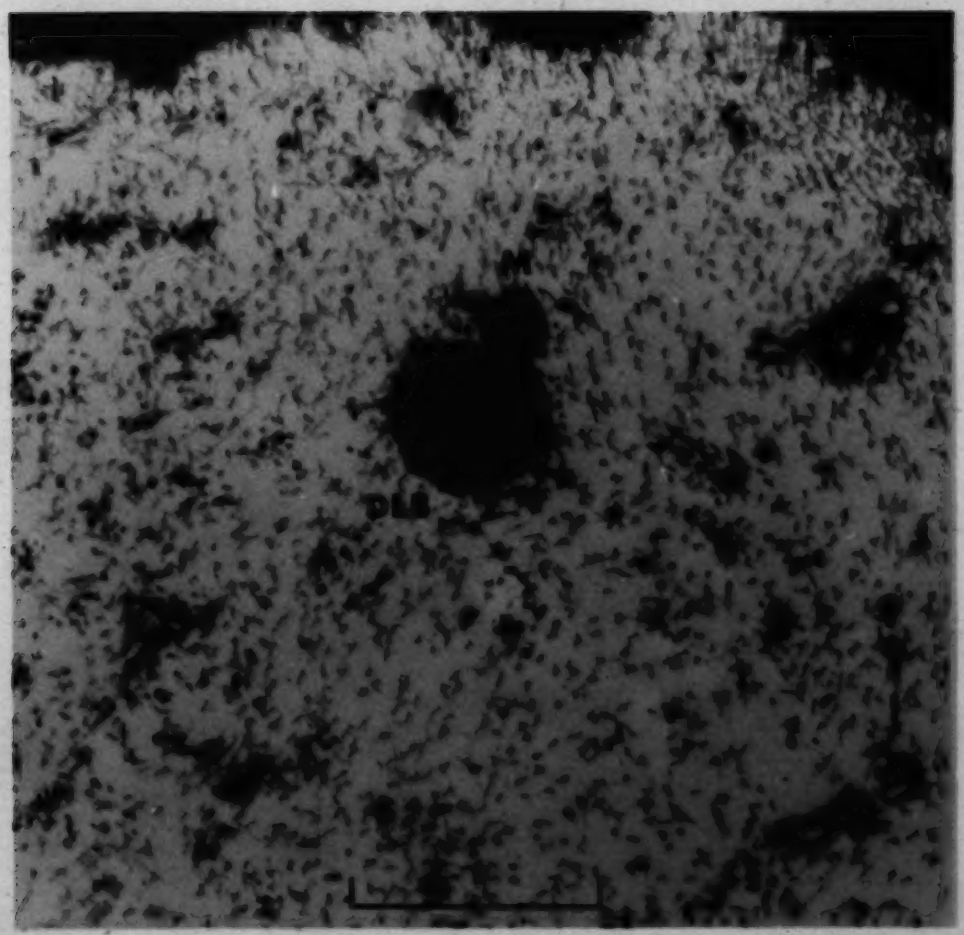
all times studied, except 1 day after the crush. However, the greatest numbers of large particles were present during that time in which most fibers had probably not made contact with their end organs.

In an attempt to identify the large organelles morphologically a large number of cross sections of normal nerve and nerve which had been allowed to regenerate between 10 days and 2 weeks were examined in the electron microscope. In this series the largest organelles present in normal nerve were mitochondria with diameters up to 0.3 µm. Very rarely in the regenerating nerve, organelles were found which had a lamellar membranous structure and diameters up to 0.5 µm (Plate 4.1). These organelles will be referred to as dense lamellar bodies (DLB) in accordance with Blümcke and Niedorf (1965a).

ORGANELLE TRANSPORT DISTAL TO THE LESION

The numbers of organelles crossing a diameter of an axon per unit time were recorded at sites 1-2 cm distal to the lesion on each of the first 4 days following the lesion. The results (Figure 4.5) show that the numbers of organelles moving in each direction declined with time. Somatopetally moving particles were present 4 days after the lesion in small numbers. Particles could be detected travelling somatofugally (away from the lesion) at 3 days. The Discussion will show that these results are not consistent with the simple assumption that an axon is a tube containing

Plate 4.1. Electron micrograph of a large, dense lamellar body (DLB), about 0.5 µm in diameter, next to a mitochondrion (M) in a cross-section of an axon examined 14 days after crushing. These organelles were found infrequently in the proximal portions of regenerating axons, but were not detected in the normal axons examined by electron microscopy. Scale bar, 1 µm.



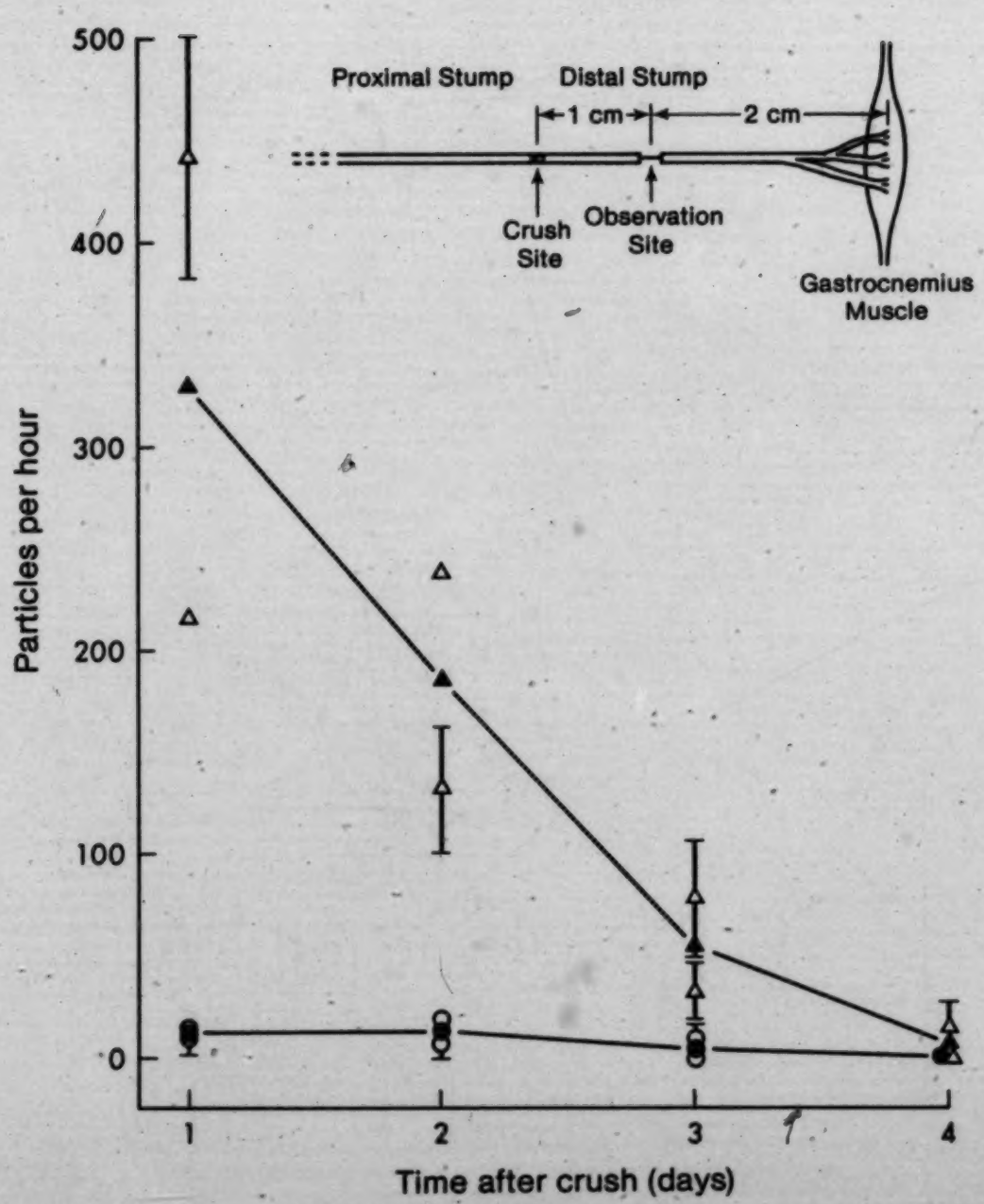


Figure 4.5. Mean numbers of somatopetally (triangles) and somatofugally (circles) travelling particles per hour in distal portions of axons (see inset) from 1 to 4 days after crushing the nerve. Two axons were studied at each time period (open symbols) and the two values were averaged for each direction (solid symbols). Vertical bars represent 95% confidence intervals for the means from experiments in which counts over 30 minute observation periods were made separately for each minute.

moving organelles whose origins are immediately interrupted when the axon is damaged.

At postoperative intervals from 34 to 100 days, when it became possible to observe transport inside the new axon sprouts, counts were made in a limited number of these developing structures. Although their diameters (about 3-10 μm) were much smaller than the established proximal portions of the axons, many of them contained extremely high numbers (>40/min) of normal appearing particles moving somatopetally at at least normal velocities. The numbers of somatofugally moving particles were also high, remaining close to the usual (approximately 10:1) proportion. It appeared from this evidence that the numbers of particles being transported proximally in the newly regenerated sprouts may be sufficient to account for the numbers measured at more proximal levels in the original segments of the axons.

DISCUSSION

THE INTERPRETATION OF REGENERATION EXPERIMENTS

The sequence of events initiated by an axonal lesion is complex and variable (Gutman et al, 1942; Grafstein, 1975; Duce and Keen, 1976; Duce, Reeves and Keen, 1976). Consequently, there is no precisely defined time at which the immediate local responses to the injury cease and those processes involved in axonal regeneration begin. It has been thought that regenerating sprouts form at an axonal lesion

after a latent period of about 2-7 days (Ramón y Cajal, 1928; Gutmann et al, 1942; Lubińska, 1964). However, there is evidence that sprouting may begin within a few hours of the damage (Perroncito, 1907, cited by Evans and Saunders, 1967) or within 24 hours of the damage (Zelená, Lubińska and Gutmann, 1968; Duce and Keen, 1976; Duce, Reeves and Keen, 1976).

In the present work, histological observations indicated that some crushed fibers had developed growth cones 24 hours after the crush. Thus, it was assumed that observations made at 1 day after the crush, and subsequently, represented the behavior of regenerating axons. This conveniently separates acute experiments on isolated segments of nerve, with maximal experiment durations of 12 hours, from those experiments in which nerve, which had been crushed at least 1 day earlier, was removed from animals for observation. Since it has been shown (Hammond, 1977) that particle transport in isolated segments of nerve is not, over a period of several hours, different from that in "intact" neurons, then differences between the control preparations used here and crushed nerve must be due to the regeneration process.

Since the present evidence indicates that the onset of regeneration is not simultaneous in all axons, then it must be expected that a variability additional to that described in Chapter 3 will have been present in the experiments. An

effort was made to eliminate inter-axonal variability by using the same isolated axon, maintained in a spinal cord-nerve-muscle organ culture, for measurements at successive time intervals. Unfortunately, repeated attempts failed to establish a culture preparation with sufficient viability for this approach, so this work is not reported here.

The consequence of having to use different control and experimental axons is that small quantitative changes in particle transport during regeneration may have been masked by the variability due to extraneous factors. Thus with respect to the mean velocity of the particles, it can only be said that no pronounced change appeared during regeneration. Small changes in particle velocity following injury could have been present and have gone undetected.

DEMONSTRABLE CHANGES IN PARTICLE TRANSPORT DURING REGENERATION

While the velocity at which particles moved showed little or no change during regeneration, evidence was obtained that the amounts of particles undergoing transport do change. The numbers of somatopetally moving particles showed a sharp decrease in the axon proximal to a crush at 1 and 2 days after the lesion. From about 1 week to 1 month after crushing these numbers returned to approximately normal levels. By 2 months, they had risen to approximately double normal values, and they remained significantly

elevated until at least 100 days. Somatofugally travelling particles rose to approximately twice their normal numbers at 8, 64 and 69 days, and were lower than normal at 100 days. In both directions, particle traffic was significantly elevated (Figure 4.3) at about 2 months after the injury. According to the functional tests and the histological estimate of the rate of regeneration, this time interval corresponded to a period when a large proportion of the axons were at the stage of re-establishing terminal connections.

These findings also indicate that the particle transport system under normal conditions is not saturated. It appears to have a reserve capacity of at least about 100 percent in the proximal direction and somewhat more in the distal direction. There must therefore be something limiting the numbers of particles to their usual levels in normal axons. Although the controlling factor(s) are not known, the present evidence suggests that at least the proximal movement appears to be systemically sensitive, and Figure 4.3 is consistent with the possibility that the distal traffic may respond to changes in the proximal flow.

The numbers of rod-shaped organelles moving in both directions increased during regeneration with the numbers moving towards the nerve terminal being greater than the numbers moving away from it. These and the foregoing findings are consistent with the idea that the changes

taking place during regeneration are largely ones in which the amounts, and perhaps kinds, of materials that are loaded onto the transport system alter while the velocity of transport remains relatively unaffected (Kreutzberg and Schubert, 1971b; Frizell and Sjöstrand, 1974d; Grafstein, 1975; Lubińska, 1975; Griffin, Drachman and Price, 1976; Ochs, 1976).

It is possible that the class of large organelles which appeared during regeneration represents a qualitative change in the type of material undergoing somatopetal transport. These organelles may be DLBs, and this would be consistent with their presence in regenerating nerve terminals (Blümcke and Niedorf, 1965a). However, since it has recently been shown that the population of somatopetally moving organelles in normal nerve is composed largely of DLBs (Smith, 1978), it is probable that the very large particles merely represent larger or more commonly occurring versions of bodies which are normally present. Similar bodies are present in large numbers in abnormal nerve (Wallace, Volk and Lazarus, 1964; Townes-Anderson and Raviola, 1978) and probably represent a form of lysosome.

CONTINUED BIDIRECTIONAL TRANSPORT IN CRUSHED AXONS

For the purposes of this discussion a simple hypothetical model of the axon as a pipe containing moving particles, is assumed. Each particle has a constant velocity but the velocities of the particles are distributed over a

range as shown in Chapter 2. In this model it follows that if the particle traffic is suddenly blocked at some point in the pipe, a change occurs in the access of particles to that portion of the pipe downstream of the blockage. An observer stationed at some point downstream of the interruption will see no change in particle traffic until the fastest particles at, and on the observer's side of, the blockage have passed the observation point. Thereafter the observer will see progressively fewer particles with progressively slower velocities. The percentage of particles remaining at a given time is determined by the distribution of velocities of the original population of particles.

Figure 4.6 shows the expected form of the decline in the numbers per unit time of somatopetally travelling particles which pass observation sites 10, 20 and 30 mm from a more distal crush. At a distance of 10 mm from the crush particle traffic should have virtually ceased at 24 hours if the assumptions made above are correct. Figure 4.2A indicates that while particle traffic is depressed at 24 hours after the lesion it is nevertheless much greater than predicted.

Similar predictions were made for particle traffic in the somatofugal direction on the distal side of the crush. Ten millimeters from the crush there should have been no detectable traffic by 15 hours after the lesion was made. The observations (Figure 4.5) showed detectable somatofugal

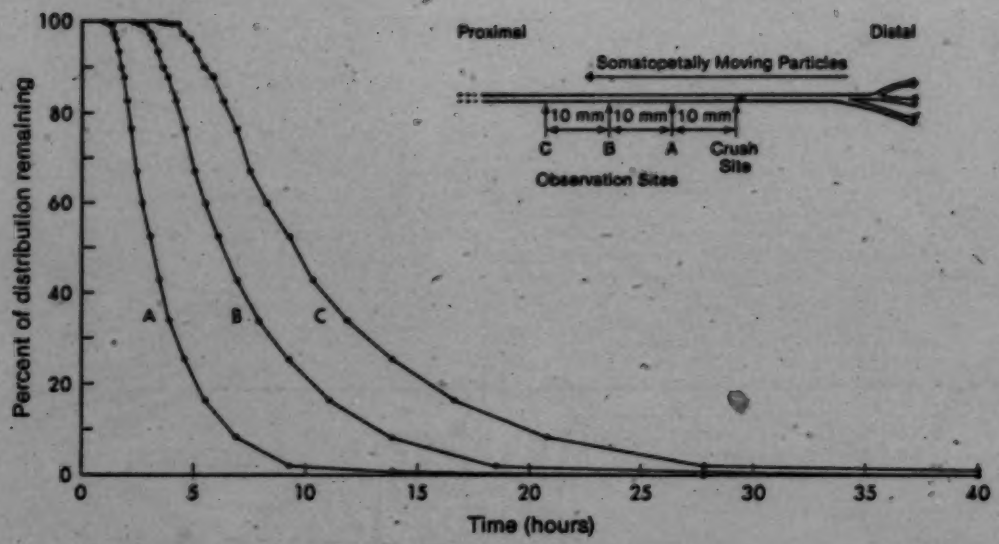


Figure 4.6. Predicted form of decline in numbers of somatopetally moving particles per minute at specified distances (see diagram) proximal to axonal interruption, according to model discussed in text. Curves based on empirically derived distribution of 400 somatopetal particle velocities in normal axons.

traffic at 3 days following the crush.

Since the velocities of particles during regeneration did not change detectably then some other aspect of the assumptions must be incorrect. It may also be noted that individual particles having velocities greater than the normal group mean could be observed in axons distal to the crush up to 3 days. The assumptions would appear to need modification to allow for a continued source of particulate material. This source might be from within the axon, as by a reversal of the direction in which particles travel or by transport of intracellular debris from the region of the crush. Or, the source might include extracellular material which enters the axon at the crushed nerve ending. These possibilities will be investigated further in Chapter 5.

The declining numbers of particles moving somatopetally in axons distal to the crush (Figure 4.5) might be consistent with the simple assumptions made above if the end organs of most of the nerve fibers were 5-10 cm distal to the crush, a distinct possibility in adult female Xenopus. However, the particles seen at 3 and 4 days should then have been travelling very slowly with velocities of about 0.1-0.2 µm/sec. As noted above, particle velocities close to 1 µm/sec could be observed at least until 3 days after the crush. This suggests that axon terminals contain a pool of organelles which may be delivered to a retrograde transport system.

CHAPTER 5

LOCAL REACTIONS AT SITES OF DAMAGE IN SINGLE AXONS

INTRODUCTION

In Chapter 4 it was noted that within the first few days after a crush lesion somatopetal particle traffic proximal to the injury, although diminished in amount, was still present despite the elimination of what may be the normal source of such particles. The origins of such organelles are particularly obscure since they are present before there is any evidence for the development of growth cones in most of the regenerating axons.

In order to propose a source for the somatopetally travelling organelles at short times following a crush lesion, the events which occur close to the lesion must be known. This chapter will describe work in which the changes in axonal structure and particle transport, as detected by light microscopy, have been investigated close to compression and crush lesions in bathing solutions of various compositions. In addition, a chance occurrence is described which indicates that particle traffic may reverse direction at sites of minimal axonal damage.

METHODS

Sciatic nerves were removed from adult female Xenopus laevis as described in Chapter 2. Dissection of single axons was carried out under Ringer solution, in chambers re-designed (Hammond and Smith, 1977) to permit the isolation and observation of sections of single axons up to several millimeters in length.

Local compressions in single axons were produced by arranging two intersecting fibers on the top of a small removable glass platform. Pressure was exerted on the intersecting fibers by a cover glass. In this way the risk of damage to the axon from contact with a harder material such as thin metal wire was avoided, and the pressure required to constrict the fiber without crushing it was less critical.

For experiments involving purposely crushed axons, a metal wire approximately 50 μm in diameter was placed across the isolated axon, and projecting slightly beyond the edges of the chamber. A cover glass was mounted and the contact point of the wire and the axon was observed under the microscope while pressure was manually applied with dissecting needles to the center of the cover glass. After the wire had produced a local crush in the axon it could be left in place, repositioned or withdrawn. The Ringer solution used during dissection could be replaced with modified solutions in the open chamber prior to crushing, or

by perfusion through the chamber at any time after the crush had been made.

The solutions used in the various experiments and their composition (mM) were as follows:

1. Ringer solution: NaCl, 112; KCl, 3; $CaCl_2$, 3; $MgCl_2$, 1.6; NaH_2PO_4, 0.45; Na_2HPO_4, 2.6; glucose, 5.
2. Isotonic potassium chloride: KCl, 122; NaH_2PO_4, 0.45; Na_2HPO_4, 2.6; glucose, 5.
3. Isotonic potassium chloride with EGTA (ethyleneglycol-bis(ß-aminoethylether)-N,N'-tetraacetic acid, dipotassium salt): KCl, 122; NaH_2PO_4, 0.45; Na_2HPO_4, 2.6; glucose, 5; EGTA, 1.0.
4. Isotonic potassium chloride with controlled calcium and magnesium ion concentrations (four solutions): a) 10^{-3} b) 10^{-4} c) 10^{-6}mM free calcium ion concentration: KCl, 122; NaH_2PO_4, 0.45; Na_2HPO_4, 2.6; glucose, 5; d) 10^{-4}mM free calcium ion concentration, with 0.03 mM free magnesium ion concentration and 8.71 mM free ATP (disodium salt): KCl, 67.5; HEPES buffer, 25; glucose, 7. The computed solution compositions in Table 1 of Fabiato and Fabiato (1975) were consulted to determine the required amounts of $CaCl_2$, EGTA, $MgCl_2$ and ATP.
5. Sodium glutamate: sodium glutamate, 120; HEPES buffer, 3.

All the solutions were gassed with oxygen before use and the pH was adjusted to 7.3-7.4.

RESULTS

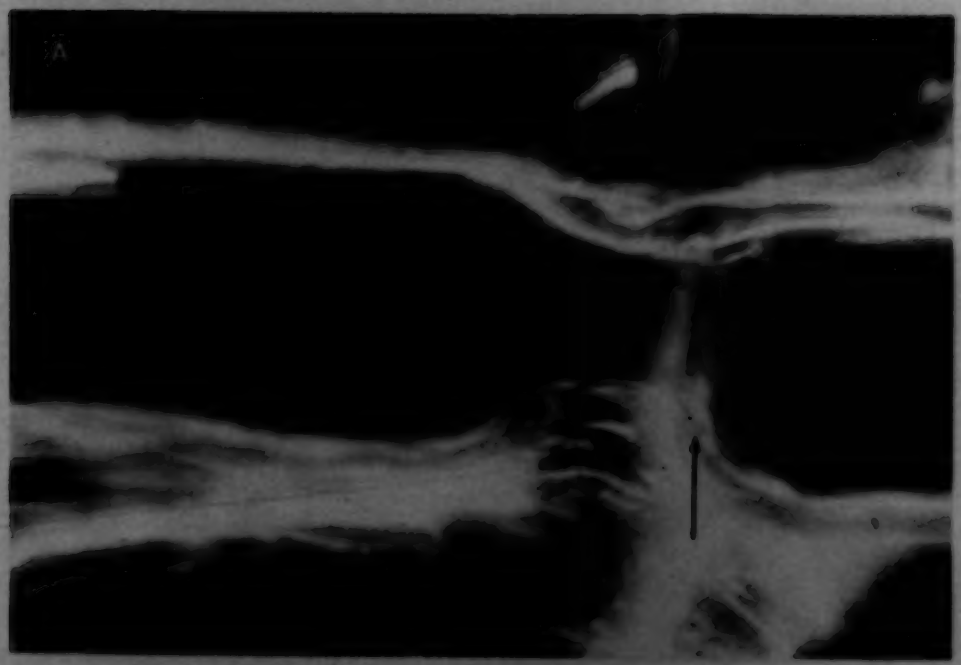
ORGANELLE TRANSPORT IN COMPRESSED AXONS

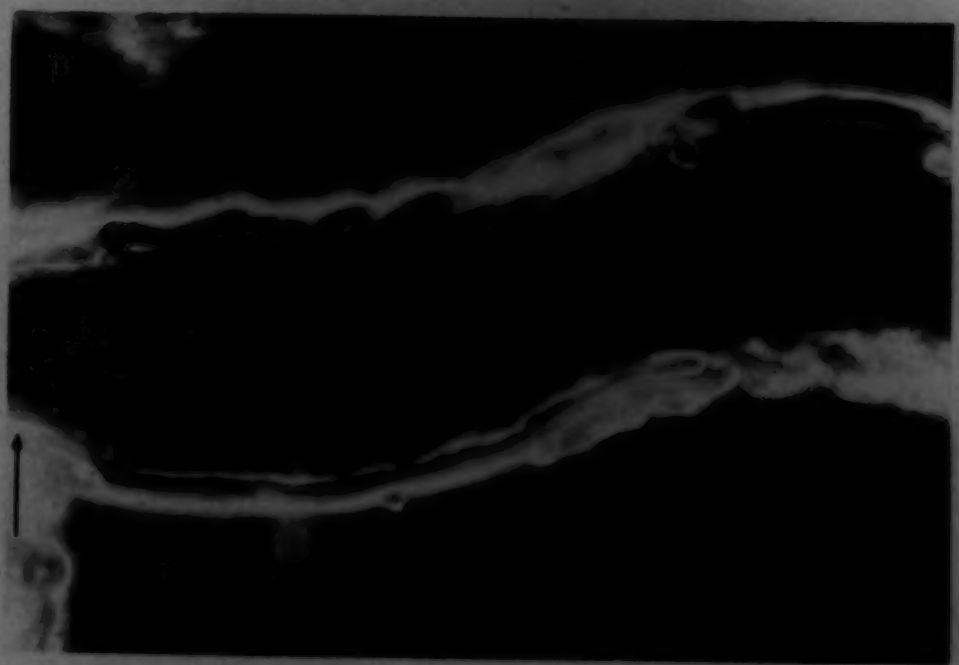
The most satisfactory procedure for preparing a single undamaged constricted axon was by arranging a second axon to cross over the first, and carefully lowering the cover glass until the desired degree of compression was achieved. With this procedure optical conditions remained favorable, and the interior of the axon immediately adjacent to the constriction was as clear and accessible for viewing as any other location along the fiber.

Within minutes of compressing an axon in Ringer solution, a zone began to appear next to the intersecting fiber in which arrested organelles were visible in elevated numbers (Plate 5.1). Some particles were motionless, while others oscillated longitudinally and irregularly over distances of a micrometer or two. The region of accumulated particles continued to gradually increase in length as more organelles continued to arrive at the constriction. In keeping with the predominant detectable traffic in the proximal direction, the accumulations were more pronounced on the distal sides of the interruptions, but small particle accumulations did appear on the proximal sides of the constrictions.

Individual particles could be followed as they approached the constriction and could be kept in sight as their progress ceased. Apart from the local increase in

Plate 5.1. Darkfield photomicrographs showing material appearing at intersection of two undamaged axons compressed against each other. Arrows indicate distal edge of compressed region. A few minutes after the fibers were compressed (A), a certain amount of material had appeared symmetrically on both sides as a direct result of the compression, and a small amount of additional material had appeared primarily on the distal side as a consequence of blocked axonal transport. After 1 hour (B), the steady collection of proximally moving material had produced a large accumulation. Magnification approximately 1000X.





numbers of organelles, the axoplasm and its visible contents appeared to remain entirely normal, as did the arrested organelles.

ORGANELLE TRANSPORT IN CRUSHED AXONS

Experiments in Ringer Solution

The experiments on crushed axons were initially performed in Ringer solution of standard composition (see Methods). Under these conditions a characteristic pattern of changes was consistently observed which was in marked contrast to the simple accumulation of organelles seen in compressed axons. For descriptive purposes six different stages were distinguished, on the basis of dynamics and morphology evident under the light microscope. These six stages are illustrated in Plate 5.2 and Figure 5.1 and are described in the captions.

The tips of the axon on both sides of the crush underwent a sequential transition through the six stages during the first 15-30 minutes after the crush, depending on the extent of the damage. Each stage, after initially developing at the crush site, progressed gradually along the axon in both directions. All the stages were apparent simultaneously by viewing along the length of the axon.

The rate of progression of the changes was documented by measuring the approximate positions of the five

Plate 5.2. Darkfield photomicrographs of progressive degenerative transformations adjacent to axonal crushes (1-6) and crushed region (bottom). Numbers correspond to stages described in Figure 5.1. Scale bar, 10 µm.

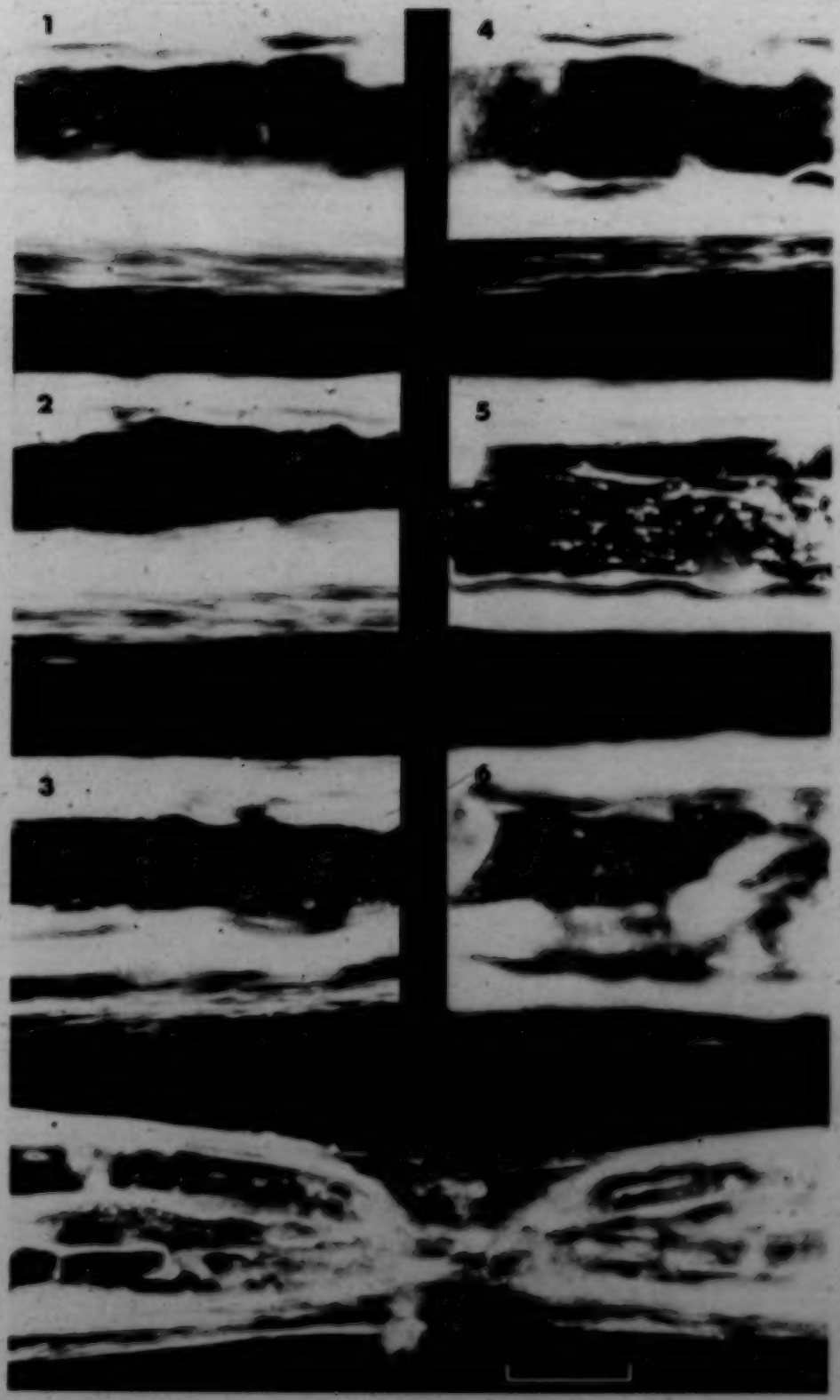

1
4
2
5
3
6

Figure 5.1. Diagrammatic representation of sequential stages of degeneration developing adjacent to axonal crushes made under Ringer and certain other solutions (horizontal distances compressed). Six stages were distinguished, based upon dynamics and morphology evident under the light microscope. Stage 1: structure and movement normal. Stage 2: structure normal, organelle movement arrested. Stage 3: initial segmentation or beading of rod-shaped structures. Stage 4: complete fragmentation into small round profiles with no apparent connection. Stage 5: development of oversized or swollen organelles or vesicles, often with the microscopic appearance of spherical bubbles. Stage 6: random milling (Brownian movement) of some particles in developing local "pockets" of axoplasm.

"interfaces" or transitions between the six stages, as a function of time after the crush. Figure 5.2 represents the results from a typical experiment, in which both the proximal and the distal progression of the changes was measured in the same axon for 4 hours subsequent to the crush.

The observations indicate that with few exceptions, all the short term gross reactions to crushing under Ringer solution were essentially complete and stabilized by the end of the first hour, and often much sooner than that. The exceptions involved the earliest change, the cessation of transport, which sometimes continued to work its way back for distances in excess of 5 mm, the observations being limited by the length of the isolated single fiber which had been dissected. The beading, fragmentation and swelling of mitochondria and perhaps other organelles tended to be very rapid, and had usually progressed almost to completion within the first 5-10 minutes. However, it usually required between about 10-30 minutes for any Brownian movement of the particles to appear.

There did not appear to be any consistent spatial or temporal differences between the changes on the proximal and on the distal sides of the injury.

It was clear from the foregoing experiments that the Ringer solution had a highly destructive effect on the structure of the axon and caused a local cessation of

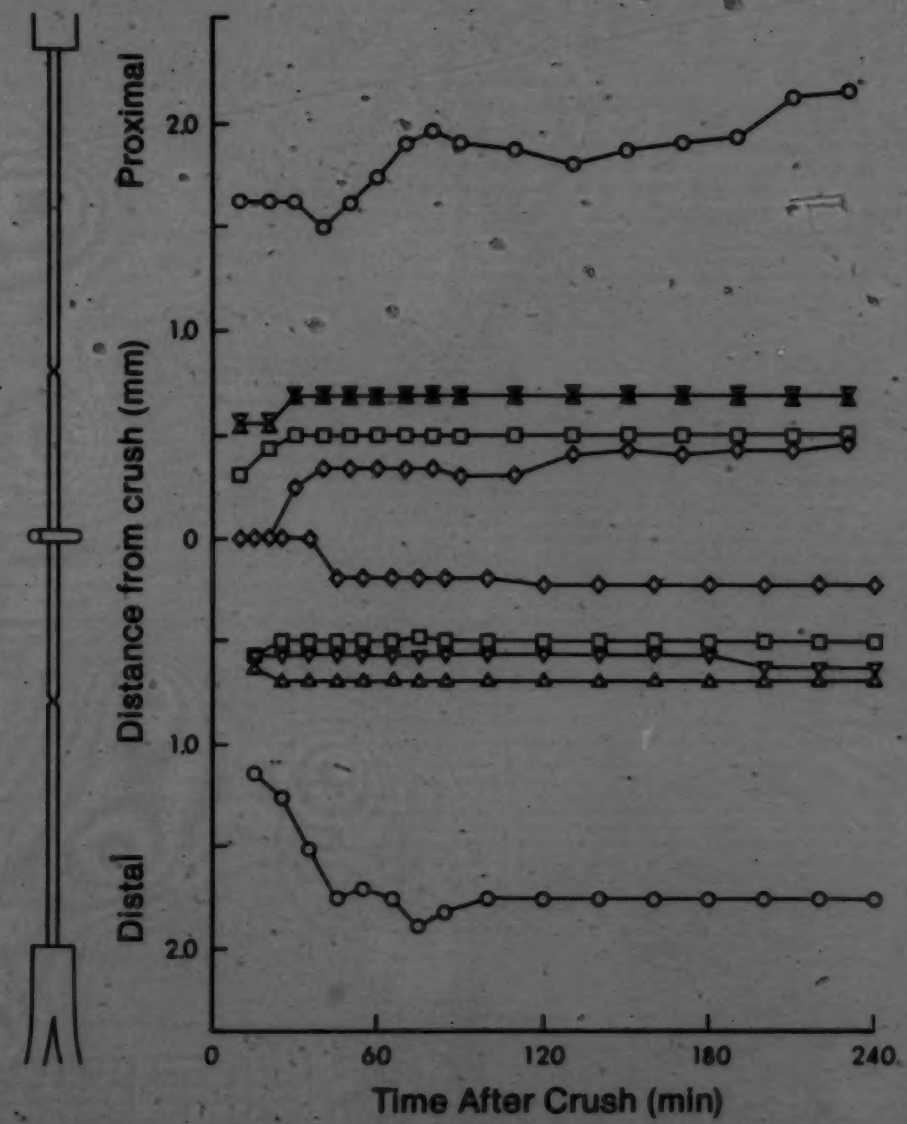


Figure 5.2. Simultaneous proximal and distal progress of respective stages of degeneration (refer to Figure 5.1) in a single axon crushed under Ringer solution. Schematic diagram of experimental preparation on left. Symbols (O, △, ▽, □ and ◇) correspond to stages 2, 3, 4, 5 and 6 of Figure 5.1, respectively, and represent the furthest detectable progress of the transformations at any given time. The effects were similar on both sides of the crush.

particle transport. In an effort to determine the causes of these changes a number of solutions were tested for their effects on crushed axons.

Experiments with Modified Solutions

(a) Isotonic potassium chloride

Compared to the effects of Ringer solution (Figure 5.2) there was a definite reduction in the progress of all five alterations when axons were crushed under isotonic potassium chloride solutions (Figure 5.3). The development of both vesiculation and the Brownian movement of particulate material were eliminated for at least the first 3 hours. The zone containing fully disintegrated rods progressed only about half as far as in Ringer solution. The initial segmentation of these structures reached the same distance as it had in Ringer solution, but the change progressed at a slower rate. Finally, there was visible evidence of organelle transport to within 0.7 mm from the crush, less than half the distance affected by the Ringer solution. The improved intra-axonal preservation by isotonic KCl suggested that axonal structure and transport might be adversely affected by the sodium, calcium or magnesium ions present in the Ringer solution.

(b) Potassium chloride plus calcium ions

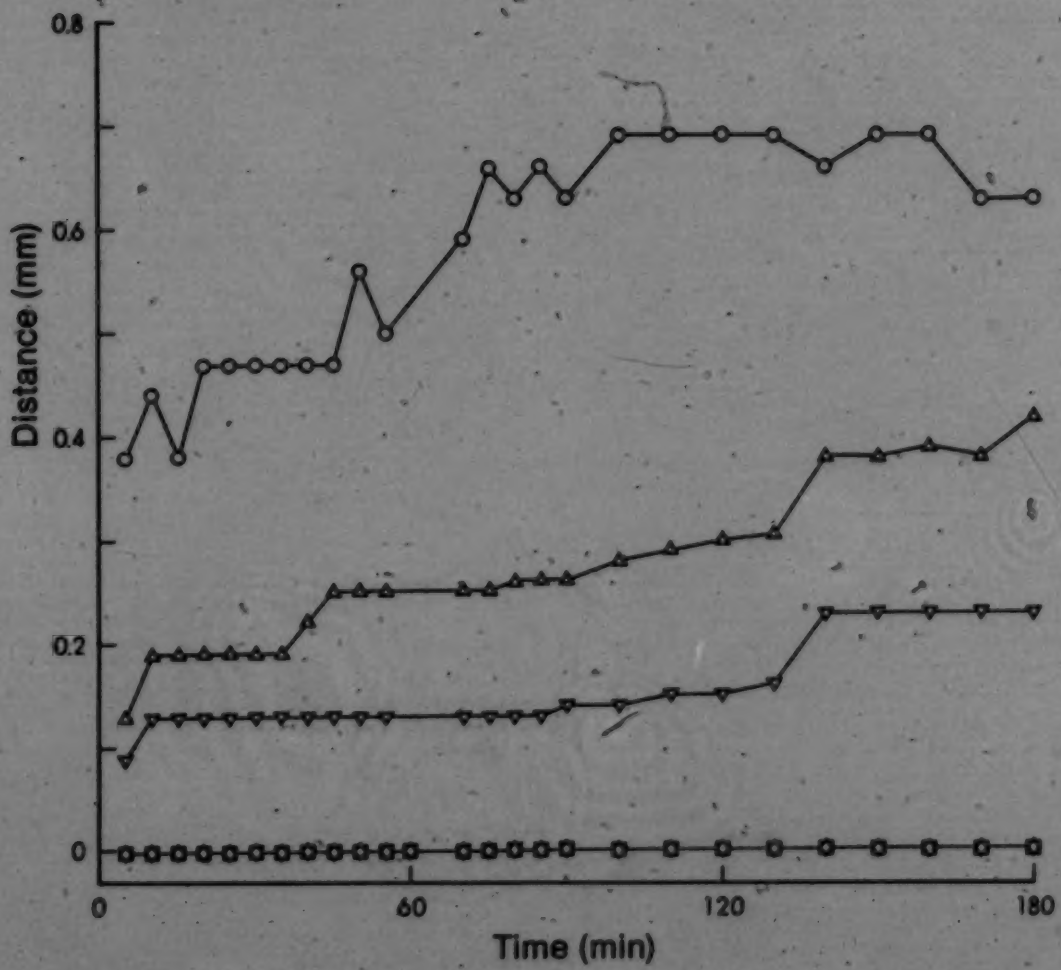


Figure 5.3. Distal progress of respective stages of degeneration in axon crushed under isotonic potassium chloride. Compared to Ringer solution (Figure 5.2), extent of stages 2(○), 3(△) and 4(▽) was much reduced while stages 5(□) and 6(◇) were eliminated, at least during the first 3 hours. Both Figures 5.2 and 5.3 are based on single experiments, but are typical of the transformations observed in several similar experiments.

Calcium chloride was added to the bathing solution at the end of every experiment done in isotonic potassium chloride, at the same (3 mM) concentration present in the Ringer solution. Each time, within 10-20 minutes, several of the previously stabilized boundaries between the zones resumed their progression away from the crush. This effect is illustrated in Figure 5.4.

In this experiment, 1-1/2 hours after crushing in isotonic KCl, all the transition zones had settled to fairly stable positions, at which time 3 mM $CaCl_2$ was added to the medium. Within 5-15 minutes of exposure to Ca^{++} a remobilization of some of the zones was observed, specifically those associated with segmentation, fragmentation and swelling of the intra-axonal organelles. Brownian movement was not observed up to 1-1/2 hours after changing solutions, but did appear within the next 2 hours. The downward shift, after addition of Ca^{++}, of the line representing the limit of normal organelle transport (Figure 5.4) indicates that particle movement was detected again in regions which had become static in the KCl solution. This could be interpreted as evidence that calcium stimulates the transport system. It is also possible, however, that this shift in the position of the boundary was artifactual, since there was also an improvement in optical conditions after changing the solutions.

(c) Potassium chloride plus EGTA

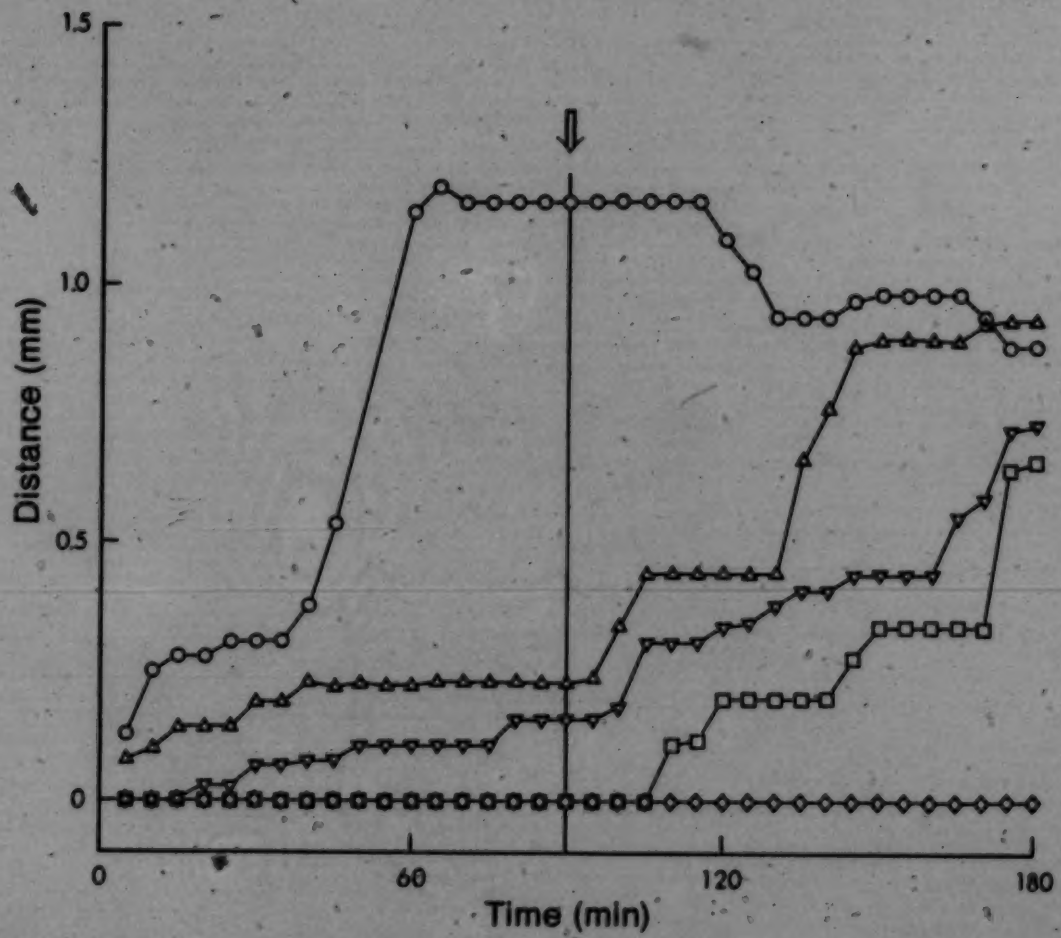


Figure 5.4. Effect of calcium on (distal) development of degenerative changes in crushed axon. Crush was initially made under isotonic potassium chloride solution after which 1 - 1/2 hours were allowed to elapse for stabilization of the boundaries between the stages (Figure 5.1). The solution was then replaced (arrow) with an equivalent solution of potassium chloride containing 3 mM calcium chloride. This treatment produced a rapid remobilization of most of the structural transformations. Symbols as for Figure 5.3. The same effect was observed in other experiments in which distances were not recorded.

Since the change from Ringer solution to isotonic KCl had reduced but not eliminated the intra-axonal disruptions, and calcium appeared to be largely responsible for these effects, an effort was made to minimize the amount of calcium present. Because the concentration of free calcium ions in axoplasm is normally about 3×10^{-7}M or less (Baker, 1972) it was possible that impurities present in normally prepared solutions might have an appreciable effect. In addition, small amounts of calcium may have been released from intra-axonal calcium sequestering organelles, such as mitochondria and smooth endoplasmic reticulum damaged by the crush. Accordingly, 1 mM EGTA was added to the isotonic KCl to chelate calcium ions free in the solution. This treatment was successful in completely preventing any of the previously described morphological changes (Figure 5.5). However, despite the elimination of any apparent morphological damage, organelle transport was not maintained.

The progressive improvement in intra-axonal preservation from Ringer solution, through isotonic potassium chloride, to potassium chloride with EGTA is evident in Figure 5.5, which summarizes all the experiments done with each solution. These figures are based upon the transition from intact to segmented mitochondria, the first optically detectable morphological abnormality, and the forerunner of the other changes. The improved structural preservation resulting from the inclusion of 1 mM EGTA in

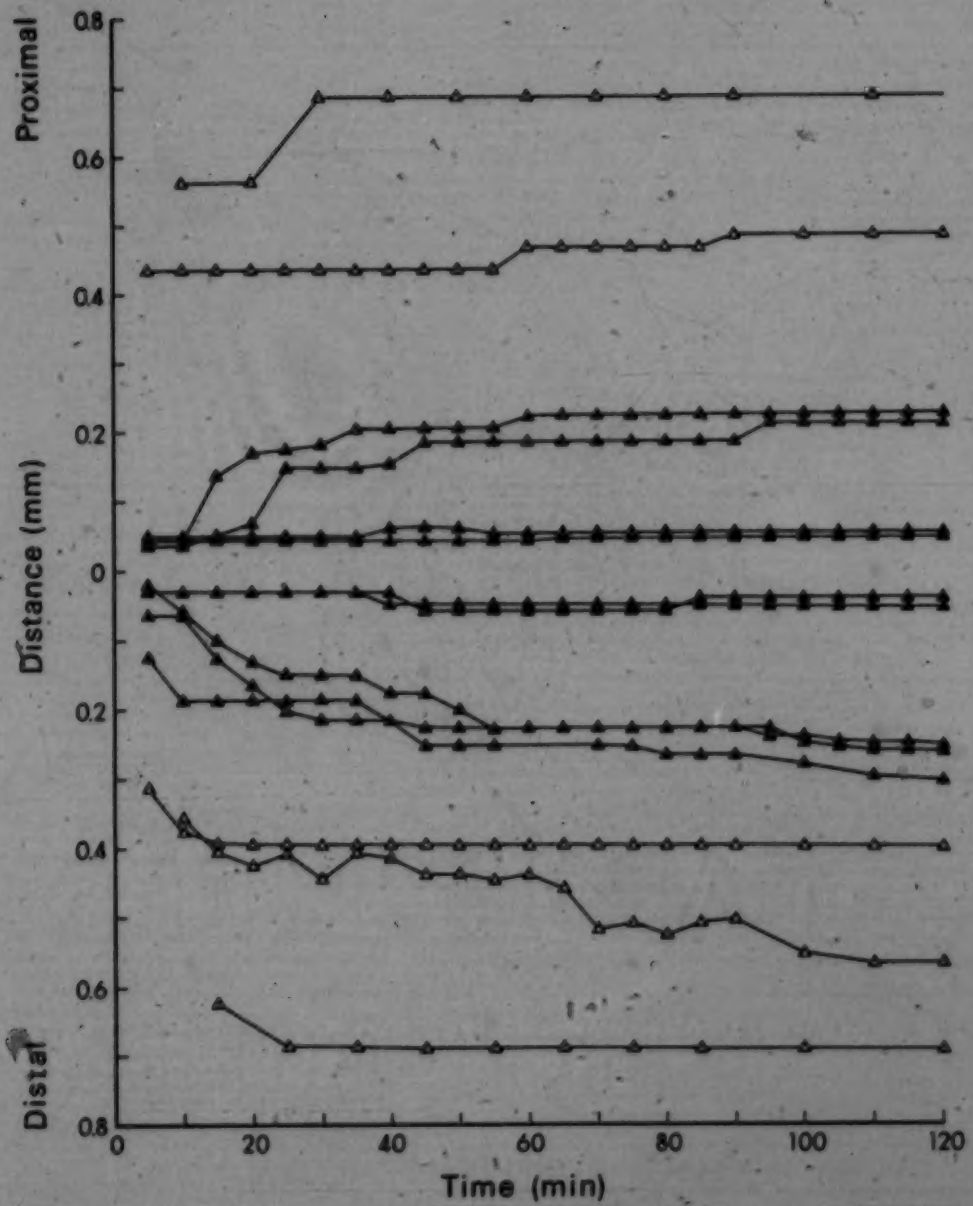


Figure 5.5 Summary of all experiments on degenerative axoplasmic changes in different solutions. Fragmentation of elongated organelles (Stage 3, Figure 5.1) was chosen for plotting as most representative single transformation. Results revealed a progressive reduction in degenerative effects from Ringer solution (clear triangles) through isotonic potassium chloride (dotted triangles) to isotonic potassium chloride with 1 mM EGTA (dark triangles), parallelling a progressive reduction in calcium ion concentrations.

the potassium chloride solution indicates that calcium (or possibly magnesium) ions have destructive effects in very low concentrations.

(d) Solutions with quantitatively controlled calcium and magnesium ion concentrations

The complete elimination of calcium by the use of isotonic KCl with 1 mM EGTA appeared to preserve the subcellular structure at the crush site but failed to maintain organelle transport, while calcium concentrations much in excess of that normally present in the axon were highly destructive. At the same time, analogies with the molecular mechanism of muscular contraction suggest that some calcium could be necessary for transport to occur (cf. Ochs, Worth and Chan, 1977). Accordingly, attempts were made to provide calcium concentrations in the physiological range by buffering the calcium to 10^{-6}, 10^{-7} and 10^{-8}M with calculated amounts of EGTA (see Methods). On the possibility (Christian, 1975) that magnesium rather than calcium might be the critical requirement, further attempts were made with one of these solutions supplemented with magnesium and ATP.

In the six axons exposed to buffered calcium ion solutions, no concentration was found, in the range from 10^{-6}M to 10^{-8}M, which was high enough to maintain organelle transport yet low enough to avoid damage to the axonal contents; in these solutions fragmentation of the

intra-axonal structures could be delayed but not prevented. Organelle transport also failed in the four axons exposed to solutions containing calcium (0.1 μM), magnesium (0.03 mM) and ATP (8.71 mM).

The failure of these solutions to maintain normal movement suggested that the elevated chloride ion concentrations present may have had an adverse effect on particle transport.

(e) Sodium glutamate

The foregoing has indicated that one of the major causes of the degenerative changes within axons close to crush lesions is likely to be an influx of calcium from the bathing medium. However, other major constituents of Ringer solution, sodium and chloride, may also contribute to the destructive effect. This view is strengthened by the recent report (Smith, - 1980) that a potassium glutamate solution will allow particle transport to continue up to a crush lesion for several hours with good preservation of axonal structure. Consequently, the effect of isotonic (120 mM) sodium glutamate solution was investigated.

Isotonic sodium glutamate solution caused morphological changes within axons close to a crush which were similar to those caused by potassium chloride solutions containing 3 mM calcium, but these changes progressed less than half as far along the axon. Particle transport did not continue up to

the lesion but was present to within 0.5 mm of the crush.

This result suggests that the sodium ion, in abnormally high concentrations, is not supportive of the structure of the axoplasm nor of particle transport. While it is possible that trace amounts of calcium in the sodium glutamate may have been responsible for the effect, a comparison with the results obtained in isotonic potassium glutamate solutions (Smith, 1980 and Table 5.1) favors sodium as being the destructive element.

Summary of the Effects of Ions Present in the Bathing Solutions

The clear difference between experiments in which axons were compressed or crushed in Ringer solution indicated that the composition of the bathing solution was not compatible with either the structure of the axoplasm or with particle transport when the solution had access to the interior of the cell. It appeared that the effects of applied solutions on the gross structure of axoplasm and on particle transport were separable.

The evidence (Table 5.1) indicated that potassium and chloride ions at concentrations of 120 mM did not cause gross axoplasmic structural changes, but that calcium ions at millimolar concentrations and 120 mM sodium did cause these changes.

Table 5.1. Effects of extracellular solutions of various compositions on particle transport and axonal structure close to an axonal crush. A. Summary of work reported in this thesis. B. Summary of work done in this laboratory by R.S. Smith. Additives to solutions in millimolar concentrations indicated by (mM), concentrations of other additives indicated in parentheses in molar units.

SOLUTION	PARTICLE TRANSPORT AT ENDING	GROSS STRUCTURAL CHANGES
A. Ringer	Very poor	Extensive
KCl	Poor	Moderate
KCl + Ca^{++}(mM)	Poor	Extensive
KCl + EGTA (mM)	Moderate	None
KCl + buffered Ca^{++} (10^{-6}, 10^{-7}, 10^{-9})	Moderate	Slight (delayed)
KCl + Ca^{++}(10^{-7}) + Mg^{++}(3×10^{-5}) + ATP (9×10^{-3})	Moderate	Slight (delayed)
Na glutamate	Moderate	Moderate
B. K glutamate	Good	Slight
K glutamate + Ca^{++}(mM)	Poor	Extensive
K glutamate + Mg^{++}(mM)	Good	Slight
K glutamate + EGTA (mM)	Poor	None

No clear evidence was obtained on those conditions necessary for maintained particle transport, but a comparison with subsequent work (Smith, 1980 and Table 5.1) suggests that 120 mM potassium is compatible with particle transport. The present series of experiments probably failed to maintain particle transport close to a crush lesion because of the presence of high chloride concentrations within the bathing solutions.

REVERSAL OF DIRECTION OF ORGANELLE TRANSPORT AT A MINIMAL LESION

The following is a description of an interesting condition, which was observed on only one occasion and entirely by chance, but which has implications for the dynamics and mechanisms of axonal transport. In an experiment originally intended for a different purpose, an isolated axon was mounted in the viewing chamber under somewhat greater tension than usual. A particular region of the axon was in some way altered but not extensively damaged, so that it became a locus for the directional reversal of particle movement (see diagram, Figure 5.6).

At the time of the first observations (about 1-1/2 hours after the frog had been killed), the particle motion everywhere in the field of view appeared to be normal. The first indications of anything unusual appeared about 1/2 hour later. It became progressively more difficult, in the central region, to distinguish proximally moving particles

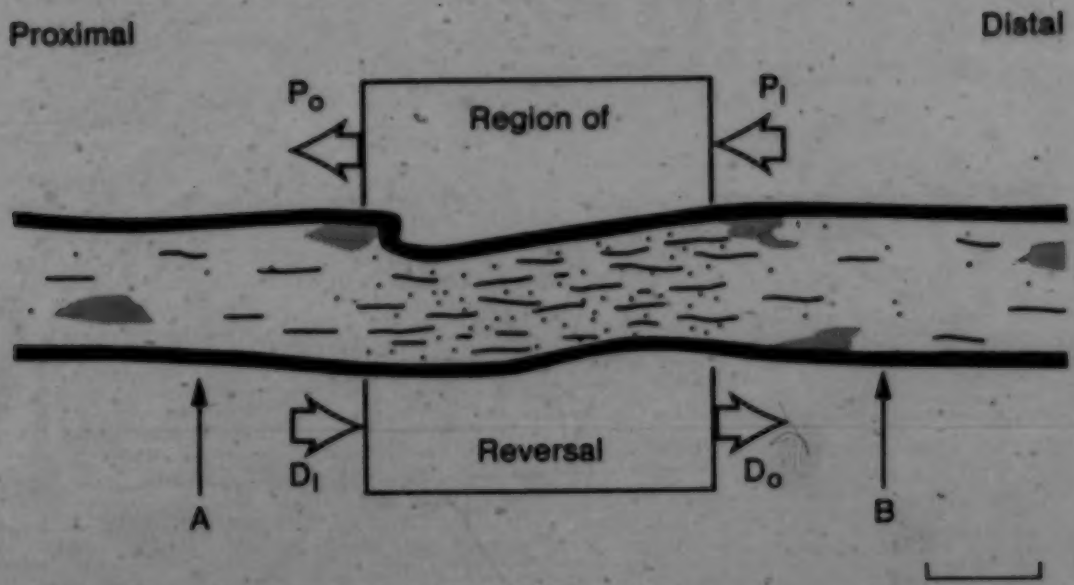


Figure 5.6. Diagram of region of axon where reversal of particle direction was documented. For explanation see text. P_I and P_0 = proximally travelling particles moving into and out of region, respectively; D_I and D_0 = distally travelling particles moving into and out of region, respectively. A and B indicate approximate positions where particle counts were made (Tables 5.1 and 5.2). Scale bar, 10 µm.

from distally moving ones, because the transient reversals often seen between saltations had increased to the extent that the backward pathlengths approximated the lengths of the forward movements (approximately 20 μm compared to a normal maximum of about 4 μm). There was a clear difference in the characteristics of particle movement between the central region and the other parts of the axon; freely moving particles approached normally in the adjacent axoplasm, but on entering the central zone they appeared to become "captured" and detained indefinitely while caught up in repetitive long, bidirectional saltations. As this local change progressed, a steady increase simultaneously took place in the numbers of particles leaving the central region on the distal side (Table 5.2). However, it was clear that the 5-fold increase in the number of particles leaving from the distal side was not accounted for by any increase in the number of particles entering the region on the proximal side. At the same time, many of the particles entering on the distal side of the region did not leave from the proximal side. It appeared that a large proportion of the particles departing distally could only be explained as being particles which had entered the central region moving proximally and had come out moving in the opposite direction.

Despite the impression that organelles reversed their direction of movement, it was impossible to track any given particle long enough to confirm that it specifically had

Table 5.2. Progressive shift in balance of proximally and distally moving particles on distal side of region of reversal. Observations made at level B in Figure 5.6.

NUMBER OF PARTICLES PER MINUTE	TIME AFTER DEATH (HOURS) 1-1/2		2		2-1/2		3	
	PROX	DIST	PROX	DIST	PROX	DIST	PROX	DIST
MINUTE 1	11	0	11	0	12	3	5	2
2	14	0	11	1	11	5	4	4
3	10	0	16	2	9	1	6	3
4	11	0	8	2	9	3	2	2
5	12	1	7	0	9	2	6	4
6	12	1	10	0	12	2	3	1
7	13	1	11	2	9	2	3	0
8	10	1	18	1	10	3	4	3
9	9	0	10	2	7	1	2	4
10	12	1	15	4	14	5	3	1
TOTAL	114	5	117	14	102	27	38	24
MEAN	11.4	0.5	11.7	1.4	10.2	2.7	3.8	2.4
P:D RATIO	22.8		8.4		3.8		1.6	

reversed direction. However, it was possible to strengthen the evidence by recording the numbers of particles entering and leaving both proximal and distal ends of the central region during alternate 1 minute periods during a 1/2 hour period (Table 5.3). Assuming no reversal and no creation or destruction of particles, it is necessary under equilibrium conditions that the number of proximally moving particles entering any portion of axon be equal to the number leaving it in the proximal direction, and similarly for the distal direction, i.e. from Figure 5.6, $P_I = P_0$ and $D_I = D_0$. If reversal does occur, neither of these equalities necessarily needs to hold, but the relationship $P_I = P_0 + D_0 - D_I$ must be satisfied.

At the time the counts were made, the process was in equilibrium or close to it, because there was no evidence of changes in particle concentration comparable to those observed in constricted axons. It was clear from the numbers obtained that this was not a normal region of axon with particles passing straight through, because P_I (55) $\neq P_0$ (31) and D_I (7) $\neq D_0$ (33). However the values did conform to the relationship allowing for reversal, P_I (55) $\approx P_0$ (31) + D_0 (33) - D_I (7).

Table 5.3. Number of particles entering and leaving each side of region of reversal (levels A and B in Figure 5.6). Counts were made during 30 one minute periods alternating between proximal and distal sides, at the end of the second hour of observation. The figures obtained are consistent with the mathematical relationship applying to a region where particles are reversing (see text), while they fail to comply with the expression for a region with particles passing through.

SIDE	PROXIMAL		DISTAL	
DIRECTION	PROXIMAL	DISTAL	PROXIMAL	DISTAL
NUMBER OF PARTICLES PER MINUTE	2	0	5	2
	3	0	4	4
	1	2	6	3
	2	1	2	2
	4	0	6	4
	1	0	3	1
	3	0	3	0
	2	0	4	3
	1	1	2	4
	2	0	3	1
	4	1	6	1
	2	2	3	3
	2	0	3	1
	1	0	4	3
	1	0	1	1
TOTAL	31	7	55	33
ABBREVIATION	P_O	D_I	P_I	D_O

DISCUSSION

LOCAL EFFECTS OF EXTRACELLULAR IONS ON DAMAGED AXONS

Experiments with crushed single axons showed that Ringer solution has a rapid destructive effect on the interior of the axon. This suggests that blood plasma and tissue intercellular fluid would probably have a similar effect on axons injured in vivo. Subsequent experimentation using various modified solutions identified some destructive components of Ringer solution, and indicated some differences between their effects on gross axoplasmic structure and on the transport of intra-axonal particles (see Table 5.1).

Gross Structural Changes

The component primarily responsible for the extensive structural damage is the calcium ion, as shown by the marked reduction of these effects with the substitution of isotonic potassium chloride for Ringer solution, the elimination of structural damage by the inclusion of EGTA in the medium, and the extension of the damaged region upon restoration of the original calcium level. A similar correlation between granular axoplasmic degeneration and degree of exposure to calcium ions was reported by Schlaepfer and Bunge, 1973 and Schlaepfer, 1974, 1977. The less pronounced degeneration in the sodium glutamate solution indicates that sodium ions are also harmful in high concentrations.

The destructive effect of calcium, in millimolar and micromolar concentrations, is consistent with the extremely low levels of this ion maintained in intact axons by sequestering or binding processes (Baker, 1972). Since mitochondria are one of the organelles which perform this function, exposure of the organelles to excessive amounts of calcium may overload the process and cause extensive alteration of the mitochondria. Functional and characteristic morphological changes, including swelling, have been reported following massive calcium ion accumulation by mitochondria in media containing 1-5 mM calcium (Greenawalt, Rossi and Lehninger, 1964; Chappell and Crofts, 1965; Lehninger, Carafoli and Rossi, 1967). The swelling is thought to be caused by some non-osmotic process (Pfeiffer, Kuo and Tchen, 1976).

Arrested Particle Transport

The transport of optically detectable particles was arrested near the crushed ends in all the solutions tested. This could have been due to the loss of some material from the ending that was not replaced by the solution, or to some change inevitably associated with the injury. However, experiments with potassium glutamate solutions (Smith, 1980) do not support these possibilities. It appears instead that maintenance of particle transport at the crushed end is largely a matter of identifying and eliminating the harmful components of the bathing solutions.

Again, the most critical factor is the calcium ion concentration. Two (not necessarily exclusive) possibilities may be mentioned for the action of calcium. If the transport process involves contractile elements dependent upon a specific narrow range of calcium ion concentrations (cf. muscle), the imbalances created by the experimental conditions may take the contractile mechanism out of its range of action. Secondly, calcium ion concentrations greater than the micromolar amounts normally present in axoplasm may disrupt subcellular organelles thought to be involved in the transport process. Microtubules, in particular, may be depolymerized by excessive concentrations of calcium ions (Schlaepfer and Bunge, 1973; Marcum et al, 1978; Nishida, 1978).

In addition to calcium, there was also evidence that both sodium and chloride ions have an unfavorable effect on particle transport. Interpretation of this evidence is complicated by the fact that trace amounts of calcium in the solutions could be capable of producing the same results. However, one notes that transport close to a crush was stopped in sodium glutamate solutions and not stopped in potassium glutamate solutions (Smith, 1980).

REVERSAL OF DIRECTION OF AXONAL TRANSPORT AT AXONAL INTERRUPTIONS

An experiment described earlier in this chapter (Figure 5.6) represents the first documented observation of a region

of axon in which reversal of particle transport occurs. Although it was not induced by an intentional axonal lesion, the reversal probably did occur at a zone of limited structural disruption. More recently, quantitative evidence of particle reversal has been obtained in axon stumps preserved by crushing the fibers in a potassium glutamate solution (Smith, 1980).

Several reports describing the reversal of axonal transport at blocked ends or natural terminals have appeared in the literature (Bray, Kon and Breckenridge, 1971; Partlow et al, 1972; Abe, Haga and Kurokawa, 1974; Bisby and Bulger, 1977; Bulger and Bisby, 1978). In all these studies, reversal of direction has been inferred from changes in the distribution of proteins, and in all cases the change in direction has been from anterograde to retrograde.

Further comparison of these results to the observations of particle reversal is restricted because of considerable differences in the time scales covered by the experimental procedures employed in most of the above studies. In the report of Bisby and Bulger (1977), however, distal crush and proximal collection ligatures were applied within hours of the injection of [^{3}H] leucine into rat spinal cords or dorsal root ganglia. It was estimated from analysis of labelled protein accumulation at the collection ligatures that the injured axons required 0.8 hours to develop the ability to reverse transport, and that once this ability was

established, protein arriving at that location in sensory and motor axons took 1.9 and 2.4 hours respectively to begin moving in the opposite direction. It was pointed out, however, that the latter values were maximal estimates, and substitution of alternative figures in the calculations can reasonably produce values in the vicinity of zero. Hence the total time required for a reversal mechanism to become established and to fully operate on incoming material could be anywhere from 0.8 to 3.2 hours. This range agrees very well with the approximately 0.5-1.5 hours taken for optically detectable particles to begin reversing in the experiment described in this chapter (Figure 5.6). Bisby and Bulger also estimated that by 28 hours after labelled protein in sensory axons reached the injury, 46% of it had been returned. This compares in the experiment reported here to the 60% of the detectable organelles which reversed direction during the half hour sampling period after the process had reached equilibrium.

In several other studies, radioactively labelled protein, transported initially in the orthograde direction, has been reported to become part of the retrogradely transported material after reaching the nerve terminals or artificially imposed interruptions. This approach has been employed as a method for studying retrograde axonal transport (Edström and Hanson, 1973b; Frizell and Sjöstrand, 1974a; Sjöstrand and Frizell, 1975; Bisby, 1976; Frizell, McLean and Sjöstrand, 1976; Bulger and Bisby, 1978). Similar

studies involving enzyme assays and histochemical protein detection were reported by Brimijion and Helland (1976) and Nagatsu et al (1976).

It appears very likely, then, that the reversal of direction of some of the material conveyed by rapid axonal transport is a consequence of certain appropriate local conditions, closely associated with the accumulation of transported material at axonal obstructions, but whose specific details are yet to be clarified. Since the above evidence indicates that reversal can begin in the first hours after interruption and continue at least for several days, this source probably constitutes the major supply of the particles moving away from experimental crushes at the early periods when growth cones were not yet developed.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

STUDY OF ORGANELLE TRANSPORT BY LIGHT MICROSCOPY

The detection of axonal organelle transport in mature single nerve fibers by light microscopy is a relatively recent accomplishment. This method allows the direct observation of many details of the transport process which are not revealed by other techniques. The approach has been utilized in the present work to establish the basic features of particle movement in normal axons, and to investigate the ways in which particle transport is affected by axonal injury and regeneration.

In Chapter 2, evidence for somatofugal and somatopetal transport of intra-axonal organelles in mature nerve fibers was presented. These observations have been confirmed by other reports (Forman, Padjen and Siggins, 1977a,b) and they establish a more complete view of organelle transport than the generally accepted version advocated by Weiss up to about 1970. (see Chapter 1).

In the excised axon segments which were studied by the present technique, particles with round or slightly elliptical profiles 0.2-0.5 μm in diameter moved somatopetally and somatofugally with comparable mean velocities of about 1 μm/sec. This velocity is close to those reported in other studies of axonal transport in

intact amphibian neurons, as cited in Chapter 2. Normally stationary rod-shaped organelles exhibited occasional longitudinal movements at similar or somewhat lower velocities. More rounded organelles moved in the somatopetal direction than in the somatofugal direction, in a ratio of roughly 10:1. No evidence of transport at a slow rate was found. While the latter two results contradicted those of Kirkpatrick, Bray and Palmer (1972) and Kirkpatrick and Stern (1973), they are confirmed by subsequent reports (Kirkpatrick and Stern, 1975; Forman, Padjen and Siggins, 1977a,b).

The rod-shaped organelles were presumed to be mitochondria and the rounded organelles were described as a heterogeneous population of mitochondria, unidentified vesicles and other bodies. Subsequent study of organelles accumulating at experimental axonal interruptions (Smith, 1980) has indicated that dense lamellar bodies, with a smaller number of mitochondria and possibly multivesicular and membrane bounded bodies, probably make up the majority of the particles moving somatopetally, while mitochondria, small vesicles and tubules, and a small number of dense lamellar bodies may account for most of the somatofugally moving particles.

In Chapter 3, the problem of high variability in experimental measurements of velocity and of numbers of particles travelling in the axons was examined in detail.

The major sources of this variability were identified and their relative importance was estimated statistically. For both velocities and the numbers of moving particles detected per unit time, the greatest variability was found between the separate measurements within individual axons. However, analysis of variance showed that differences between individual axons also constituted an important and statistically significant source of variability. This finding was contrary to reports based on smaller sample sizes (Forman, Padjen and Siggins, 1975a,b, 1977a). The variation between different animals was small and not statistically significant. It was concluded that due to the inter-axonal variability experimental effects are best studied within individual axons, and the advantage of this approach may well justify the effort involved. However, in cases where this is not possible, the sampling error can be minimized by employing a large number of measurements per axon and a fairly large number of axons. The results indicated that in general it is not necessary to sample over a large number of animals.

The quantity of material transported along an axon per unit time is determined by the product of the quantity of material per unit volume, the cross sectional area of the axon, and the velocity at which the material moves. Neither velocity nor cross sectional area was correlated with the number of particles which passed an observation point per unit time in axons in the range of diameters typically

employed (approximately 13-22 μm).

Experiments with locally crushed axons (Chapter 4) showed that some organelles continued to move in both directions at all times during regeneration. There was a substantial reduction in the number of proximally moving organelles immediately following the injury, but the numbers rapidly increased to about their normal range at 4 days. This was the same time at which large numbers of regenerating sprouts began to appear at the nerve stump. No appreciable change in particle velocity was observed, but other changes, such as increased movement of elongated organelles, and the appearance of unusually large rounded organelles, were found. These alterations appeared to last throughout the entire regeneration period.

Factors influencing these changes were investigated further (Chapter 5) in a study of events occurring in the immediate vicinity of axonal interruptions. Historically there has been a strong polarization of opinion between two interpretations of the origin of intra-axonal material which appears adjacent to lesions. One view is that this material represents a local transformation of material which is already present, and the other is that the material arrives by axonal transport (these ideas are reviewed in Chapter 1). This dichotomy has tended to obscure the fact that it is not necessary to consider the two explanations as mutually exclusive. The results from the present experimental

approach provide support for both possibilities.

The type of material appearing at an interruption was found to depend on the specific nature of the interruption, the ionic composition of the surrounding fluid, and the extent of its access to the interior of the axon. At constrictions producing little or no structural damage a major part of the material arrives through blocked transport, while local transformations are relatively limited. Interruptions with significant damage may produce considerable transformation, depending upon the composition and degree of access of the surrounding fluid. The calcium ion is a component of blood plasma, intercellular fluid and conventional laboratory salt solutions, which, in millimolar concentrations, can produce gross structural intracellular changes. Sodium ions also appear to have a disruptive effect on intra-axonal structure in concentrations of about 120 mM.

Transported material may become "superimposed" on the original stationary structures, thereby supplying additional material for subsequent transformation, or transport may quickly be arrested at variable distances away from the injury. Any of calcium, sodium or chloride ions, in concentrations substantially higher than their normal inter-axonal levels, appear to cause the arrest of organelle movement at some distance from the point of entry of the ions.

Crushing whole nerves, as practiced in most of the earlier studies, could result in uncontrolled variation in the extent of damage and access of extracellular fluid to the individual axons. By demonstrating the diversity of potential responses, direct microscopic observation of constricted and damaged single axons has provided a new perspective on the events leading to the observed accumulations of material adjacent to lesions. Both arrested transport and local transformation may contribute to the result, with their relative importance being strongly influenced by the exact conditions at the interruption. This dependence may account for the wide variety of results and interpretations presented in the earlier studies.

SUMMARY OF CHANGES IN PARTICLE TRANSPORT IN RELATION TO CONDITIONS AT CRUSHED ENDS

From a consideration of the combined results of Chapters 4 and 5, the general idea emerges that the numbers of organelles transported in normal, damaged and regenerating axons is closely related to the conditions existing at the axon terminals. The changes in particle transport observed at the progressive stages following axonal interruption are discussed below as they relate to the changing situation at the axon terminals, and are diagramed schematically in Figure 6.1. From a functional point of view, that part of the axon which constitutes the

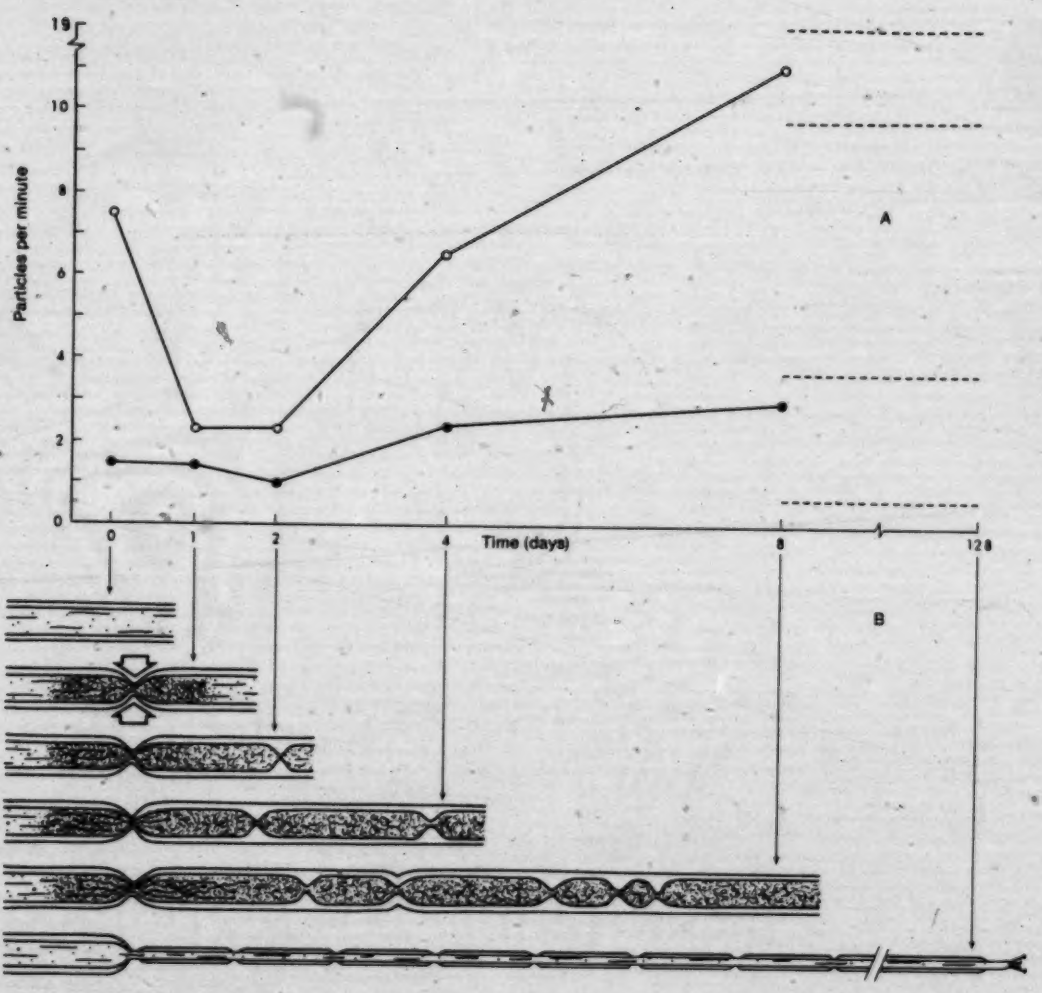


Figure 6.1. Summary of (A) changes in mean number of particles transported per minute on proximal side of axonal crush, and their relationship to (B) the changes progressively occurring at the crush site (arrows) and in the regenerating fiber. Details discussed in text. Proximal movement represented by open circles, distal movement by solid circles. Schematic drawings not to scale.

"terminal" varies according to experimental conditions and the stage of recovery of the axon.

The initial result of axonal injury is the entry of extracellular medium and its diffusion for some distance along the fiber. Exposure of the interior of the axon to elevated concentrations of ions commonly present in intercellular fluid, principally calcium, sodium and chloride ions, produces a rapid progression of degenerative changes, ending in a disorganization of the axoplasm within about half a millimeter on each side of the lesion, and cessation of particle movement in the adjacent 1-5 mm. Beyond these regions, particle transport continues normally, but does not result in the distinct accumulations of organelles appearing in axons interrupted by less destructive techniques. This is presumably because, within the duration of the experiments (2-4 hours), the boundary between normal axoplasm (region 1, Figure 5.1) and axoplasm in which particle transport had ceased (region 2, Figure 5.1) does not occupy a fixed position. It recedes from the injury at a rate which is faster than the rate of accumulation of organelles. It is not presently known whether the boundary subsequently stabilizes at its level of maximum progress, or whether the region in which movement was arrested (region 2, Figure 5.1) later returns to an active state. However, the level of origin of the regenerating sprouts (discussed below) suggests that activity may be restored.

By 24 hours, marked differences appear in the responses of the proximal and distal stumps to crushing, and two entirely different courses of events are subsequently followed. In the isolated distal stump, transport gradually declines over a period of about 4 days. In the proximal stump, organelle transport continues in both directions. A sharp reduction occurs in the numbers of proximally moving particles as a result of interruption of the supply from the more distal part of the neuron, but the axon does continue transporting a reduced number of particles.

The first regenerating sprouts emerge at about 2 days after the injury. In silver stained axons, the sprouts did not appear to originate at the edge of the crush, but about 0.4-0.5 mm back from the edge. This closely corresponds to the border between the inactive but normal appearing (region 2, Figure 5.1) and severely disrupted (regions 3-6, Figure 5.1) axoplasm in the living fibers. If in fact the nerve sprouts did appear at this level rather than between regions 1 and 2 (Figure 5.1) then this would imply a return of function to the region of arrested transport at some time between 4 hours and 2 days following the crush.

At the same time that the sprouts begin to extend from the intact axoplasm, the numbers of proximally moving particles start to increase (Figure 6.1). During the next few days these sprouts extend further and new ones appear, while the proximal particle traffic steadily recovers to its

normal range. The correspondence between these two processes could be accounted for by the pronounced pinocytotic activity observed at axonal growth cones (Lewis, 1945; Hughes, 1953; Nakai, 1956; Pomerat et al, 1967; Leestma and Freeman, 1977). This suggestion is supported by reports of direct observation of somatopetal movement of pinocytosed material (Hughes, 1953; Nakai, 1956). In addition, it is known that exogenous proteins are taken up at damaged, regenerating and intact axon tips, subsequently appearing in membrane-bounded bodies at more proximal levels of the axons and in the cell bodies (Kristensson and Sjöstrand, 1972; Kristensson and Olsson, 1974; La Vail and La Vail, 1972, 1974). At the longer time periods (from 34 to 100 days), observations reported in Chapter 4 indicated that the numbers of somatopetally moving particles detected distal to the lesions in the newly regenerated sprouts may have been great enough to account for the numbers observed at levels proximal to the lesions, in the original mature portions of the nerve fibers.

Somatofugal transport remains relatively unaffected shortly after the crush, when somatopetal transport is at a minimum. However it does show an increase to about twice its normal level in the vicinity of about 4-8 days, and again around 64-69 days. Examination of axons at periods up to 128 days after crushing suggests that both proximal and distal transport are maintained at least at normal levels throughout the later stages, when the regenerating fibers

are lengthening and re-establishing synaptic contacts, and perhaps even later when they are growing in diameter.

Figure 6.1A is interpreted as representing a transition between two separate but overlapping processes. The effect on transport during the first day or so, which is confined to particles moving in the proximal direction, is the direct result of local blockage, and does not involve the rest of the cell. But by 3 or 4 days, the cell body has presumably experienced a distinct decrease in the material arriving by somatopetal transport. Since material travelling at the average velocity would require approximately 1 day to reach the cell body and another day to return to the crush, there has probably been sufficient time for the soma to respond and become involved in determining the observed quantitative and qualitative changes in somatofugal transport. At the opposite pole of the cell, extensive changes have taken place in the structure and function of the axon terminal, influencing the content of the somatopetal flow. The characteristics of axonal transport beyond this period, then, reflect the participation of the entire cell in the regenerative process.

The observed changes in transport during regeneration appear to involve only the numbers and nature of the particles in the flow; there was no evidence for changes in velocity.

CONTINUATION OF TRANSPORT AFTER INTERRUPTION OF NORMAL SUPPLY

It was established in Chapter 4 that somatopetal organelle transport persisted in crushed axons in spite of their isolation by the crush from their normal source of supply from the more distal parts of the neuron. Detailed consideration of the specific regions developing at crushed endings (Figure 5.1) allows an explanation to be advanced for this unexpected observation. Again, the observed changes in particle transport can be accounted for on the basis of the changing conditions at the newly created terminals. Five possibilities may be distinguished, whose relative importance probably varies with the characteristics of the terminals at the respective stages of recovery from the injury.

Particles from the Unaffected Region

At about 1 day after crushing, all the unaffected somatopetally moving particles (region 1, Figure 5.1) with velocities in the measurable range (Figure 3.1) would be expected to have moved past the observation points (Figure 4.6). However, it would still be possible for rod-shaped particles, or for rounded particles oscillating about a fixed position at the time of the crush, to appear at the observation locations after the calculated time limit. In addition, a proportion of the particles which were making

net progress at the time of the crush could subsequently be delayed for indefinite periods by temporarily entering into an oscillating movement pattern.

Particles from the Stationary Region

In the experiments performed under Ringer solution, a distinct 1-5 mm zone was consistently formed between the extensively degenerated and the unaffected axoplasm (region 2, Figure 5.1). While no movement was observed in this region (either saltatory or Brownian), it appeared to remain structurally intact. In particular, the destruction of mitochondria, which are known to take up calcium ions from the surrounding axoplasm (Baker, 1972), was limited to about 0.4-0.7 mm from the lesion. It may be suggested therefore that mitochondria (Carafoli, 1974) and other calcium-sequestering organelles (Stoeckel et al, 1975) may eventually restore the calcium levels in this region to normal values. Axonal microtubules, whose depolymerization and repolymerization are rapid (Weisenberg and Deery, 1976) and may be regulated by calcium ion concentration (Marcum et al, 1978; Nishida, 1978), could then reappear, allowing particle transport, which may depend upon microtubules (Cooper and Smith, 1974; Heslop, 1975; Hammond and Smith, 1977) to resume.

Particles which were travelling somatofugally at the time of the crush might also contribute to the continued

somatopetal traffic. Since the factors controlling the direction of axonal transport are not presently understood, it may be possible for particles to resume moving oppositely to their previous direction.

Reversal of Somatofugally Moving Particles

While the eventual resumption of movement in the opposite direction could be considered an indirect and limited form of particle reversal, there is evidence for a more direct form, which appears to be a characteristic reaction in regions of relatively undisturbed axoplasm adjacent to axonal interruptions. This process was discussed in detail in Chapter 5, and it was concluded that it probably represents a major supply of somatopetally moving particles, beginning about 1 or 2 days following the injury.

Injury-generated Particulate Material

Another potentially important source of particulate material could be the damaged axoplasm in the extensively denegerated region of the axon immediately adjacent to the crush (regions 3-6, Figure 5.1). However, there would be difficulties involved in bringing material from this location into a region of active transport (region 1, Figure 5.1). In the early stage these two regions are separated by a 1-5 mm length of axon (region 2, Figure 5.1) in which no movement is seen. Within a day or two, by the time that

normal functions may have been restored in this zone, the axon endings have formed discrete sprouts, separating the interior of the axon from its degenerated former contents. If this material does make any contribution to the somatopetal traffic, it would seem to be restricted to a relatively short time period between these two stages.

Material Originating Externally

Since somatopetal particle traffic in interrupted axons recovers to at least its normal level by about 4-8 days and appears to remain at normal or higher than normal levels during the entire regeneration period (Figures 4.2, 4.3 and 5.7), some source for these particles must become established which is not an immediate and transient consequence of the injury. Continual reversal of distally transported material at the crush location or at the growing tips could provide an ongoing source, but the somatofugal particle movement does not appear to be adequate to account for all of the ascending particles. Furthermore, on the molecular level, much of the material transported distally would be expected not to return, but to be incorporated into the growing axon.

The remaining major possible source for ascending particles is suggested by the pinocytotic activity associated with neuronal growth cones. This suggestion is supported by observations from Chapter 4 and by a number of

reports of uptake and somatopetal movement of external material, and was discussed previously in this chapter. It seems reasonable to conclude, therefore, that the composition of the sustained somatopetal particle flow proximal to a lesion progressively changes, not only quantitatively, but also with respect to the nature and the origin of the transported particles, as a function of the changing conditions at the axon endings. More generally, the present research provides evidence that the somatopetal transport of a large proportion of axonal organelles in normal, damaged and regenerating neurons is related primarily to events at the axon terminals. Since changing conditions at the ends of the axons can effect considerable changes in the nature of the material transported proximally, there appears to be ample opportunity for the axon terminals to influence the synthetic and other processes occurring in the cell bodies (cf. Cragg, 1970; Watson, 1974).

REFERENCES

Abe, T., Haga, T. and Kurokawa, M., 1974. Retrograde axoplasmic transport: its continuation as anterograde transport. F.E.B.S. Letters 47:272-275.

Austin, L., Bray, J.J. and Young, R.J., 1966. Transport of proteins and ribonucleic acid along nerve axons. J. Neurochem. 13:1267-1269.

Baer, G.M., Shanthaveerappa, T.R. and Bourne, G.H., 1965. Studies on the pathogenesis of fixed rabies virus in rats. Bull. Wld. Hlth. Org. 33:783-794.

Baker, P.F., 1972. Transport and metabolism of calcium ions in nerve. Prog. Biophys. Mol. Biol. 24:177-223.

Banks, P., Mangnall, D. and Mayor, D., 1969. The re-distribution of cytochrome oxidase, noradrenaline and adenosine triphosphate in adrenergic nerves constricted at two points. J. Physiol. 200:745-762.

Barondes, S.H., 1968. Further studies on the transport of protein to nerve endings. J. Neurochem. 15:343-350.

Barondes, S.H., 1969. Axoplasmic transport. In Lajtha, A. (Ed.), Handbook of Neurochemistry, Volume II. Structural Neurochemistry, pp. 435-446. Plenum Press, New York.

Barondes, S.H., 1974. Synaptic macromolecules: identification and metabolism. Ann. Rev. Biochem. 43:147-168.

Berlinrood, M., McGee-Russell, S.M. and Allen, R.D., 1972. Patterns of particle movement in nerve fibers in vitro - an analysis by photokymography and microscopy. J. Cell Sci. 11:875-886.

Bisby, M.A., 1976. Orthograde and retrograde axonal transport of labeled protein in motoneurons. Exp. Neurol. 50:628-640.

Bisby, M.A., 1977. Axonal transport of protein in regenerating motor and sensory axons of the rat. Physiol. Can. 8:29.

Bisby, M.A. and Bulger, V.T., 1977. Reversal of axonal transport at a nerve crush. J. Neurochem. 29:313-320.

Blümcke, S. and Niedorf, H.R., 1964. Elektronenoptische untersuchungen über die feinstruktur der "neurofilamente" in der normalen und regenerierenden peripheren nervenfaser. Beitr. Path. Anat. 130:133-157.

Blümcke, S. and Niedorf, H.R., 1965a. Elektronenmikroskopische untersuchungen an lamellenkörpern im regenerirenden peripheren nerven. Beitr. Path. Anat. 131:38-62.

Blümcke, S. and Niedorf, H.R., 1965b. Elektronenoptische untersuchungen an wachstumsendkolben regeneriender peripherer nervenfasern. Virchows Arch. Path.Anat. 340:93-104.

Blümcke, S., Niedorf, H.R and Rode, J., 1966. Axoplasmic alterations in the proximal and distal stumps of transsected nerves. Acta Neuropath. 7:44-61.

Blume, R. and Scharf, J.H., 1964. Histologische, histotopochemische und statistische Untersuchungen über Länge und Verteilung der Mitochondrien in der peripheren segmentierten Nervenfaser und ihren Hüllzellen des N. intercostalis bei Bos taurus. Acta Histochem. 19:24-66.

Bodian, D., 1964. An electron-microscopic study of the monkey spinal cord. Bull. Johns Hopkins Hosp. 114:13-119.

Bodian, D. and Howe, H.A., 1941. Experimental studies on intraneural spread of poliomyelitis virus. Bull. Johns Hopkins Hosp. 68:248-267.

Bondy, S.C. and Madsen, C.J., 1971. Development of rapid axonal flow in the chick embryo. J. Neurobiol. 2:279-286.

Boyle, F.C. and Gillespie, J.S., 1970. Accumulation and loss of noradrenaline central to a constriction on adrenergic nerves. Europ. J. Pharmacol. 12:77-84.

Bray, J.J. and Austin, L., 1969. Axoplasmic transport of ^{14}C proteins at two rates in chicken sciatic nerve. Brain Res. 12:230-233.

Bray, J.J., Kon, C.M. and Breckenridge, B.M., 1971. Reversed polarity of rapid axonal transport in chicken motoneurons. Brain Res. 33:560-564.

Breuer, A.C., Christian, C.N., Henkart, M. and Nelson, P.G., 1975. Computer analysis of organelle translocation in primary neuronal cultures and continuous cell lines. J. Cell Biol. 65:562-576.

Brimijoin, S., 1975. Stop-flow: A new technique for measuring axonal transport, and its application to the transport of dopamine-B-hydroxylase. J. Neurobiol. 6:379-394.

Brimijoin, S., 1977. A histofluorescence study of events accompanying accumulation and migration of norepinephrine within locally cooled nerves. J. Neurobiol. 8:251-263.

Brimijoin, S. and Helland, L., 1976. Rapid retrograde transport of dopamine-ß-hydroxylase as examined by the stop-flow technique. Brain Res. 102:217-228.

Brimijoin, S. and Wiermaa, M.J., 1977a. Direct comparison of the rapid axonal transport of norepinephrine and dopamine-ß-hydroxylase activity. J. Neurobiol. 8:239-250.

Brimijoin, S. and Wiermaa, M.J., 1977b. Rapid axonal transport of tyrosine hydroxylase in rabbit sciatic nerves. Brain Res. 121:77-96.

Bulger, V.T. and Bisby, M.A., 1978. Reversal of axonal transport in regenerating nerves. J. Neurochem. 31:1411-1418.

Burdwood, W.D., 1965. Rapid bidirectional particle movement in neurons. J. Cell. Biol. 27:115A.

Carafoli, E., 1974. Mitochondrial uptake of calcium ions and the regulation of cell function. pp. 89-109 in Smellie, R.M.S. (Ed.). Biochemical Society Symposia, Number 39. Calcium and Cell Regulation. The Biochemical Society, London.

Carlin, R.K., 1977. Nonribosomal incorporation of amino acids into the troponin-like protein from synaptosomes. J. Neurochem. 28:355-360.

Carlsson, C.-A., Bolander, P. and Sjöstrand, J., 1971. Changes in axonal transport during regeneration of feline ventral roots. J. Neurol. Sci. (Amst.) 14:75-93.

Ceccarelli, B., Hurlbut, W.P. and Mauro, A., 1972. Depletion of vesicles from frog neuromuscular junctions by prolonged tetanic stimulation. J. Cell Biol. 54:30-38.

Chappell, J.B. and Crofts, A.R., 1965. Calcium ion accumulation and volume changes of isolated liver mitochondria. Calcium ion-induced swelling. Biochem. J. 95:378-386.

Cheah, T.B. and Geffen, L.B., 1973. Effects of axonal injury on norepinephrine, tyrosine hydroxylase and monoamine oxidase levels in sympathetic ganglia. J. Neurobiol. 4:443-452.

Christ, J.F., 1962. The early changes in the hypophysial

neurosecretory fibres after coagulation. pp. 125-147 in Heller, H. and Clark, R.B. (Eds.). Neurosecretion. Academic Press, London.

Christian, C.N., 1975. Organelle movement in the neurites of cultured dorsal root ganglion cells is dependent on magnesium and blocked by calcium. Neurosci. Abstr. 1:801 (Abstr. no. 1228).

Cook, M.L. and Stevens, J.G., 1973. Pathogenesis of herpetic neuritis and ganglionitis in mice: evidence for intra-axonal transport of infection. Infectn. Immun. 7:272-288.

Cook, M.L. and Whitlock, D.G., 1975. Axoplasmic transport in the toad Bufo marinus. Brain Res. 96:247-265.

Cooper, P.D. and Smith, R.S., 1974. The movement of optically detectable organelles in myelinated axons of Xenopus laevis. J. Physiol. 242:77-97.

Cowan, W.M. and Cuénod, M. (Eds.), 1975. The Use of Axonal Transport for Studies of Neuronal Connectivity. Elsevier, Amsterdam.

Cragg, B.G., 1970. What is the signal for chromatolysis? Brain Res. 23:1-21.

Crossland, W.J., Currie, J.R., Rogers, L.A. and Cowan, W.M., 1974. Evidence for a rapid phase of axoplasmic transport at early stages in the development of the visual system of the chick and frog. Brain Res. 78:483-489.

Dahlström, A., 1965. Observations on the accumulation of noradrenaline in the proximal and distal parts of peripheral adrenergic nerves after compression. J. Anat. 99:677-689.

Dahlström, A., 1967a. The transport of noradrenaline between two simultaneously performed ligations of the sciatic nerves of rat and cat. Acta Physiol. Scand. 69:158-166.

Dahlström, A., 1967b. The effect of reserpine and tetrabenazine on the accumulation of noradrenaline in the rat sciatic nerve after ligation. Acta Physiol. Scand. 69:167-179.

Dahlström, A., 1971. Axoplasmic transport (with particular respect to adrenergic neurons). Phil. Trans. Roy. Soc. Lond. B 261:325-358.

Dahlström, A. and Fuxe, K., 1964a. A method for the demonstration of adrenergic nerve fibres in peripheral nerves. Zeit. Zell. Mikr. Anat. 62:602-607.

Dahlström, A. and Fuxe, K., 1964b. A method for the demonstration of monoamine-containing nerve fibres in the central nervous system. **Acta Physiol. Scand.** 60:293-294.

Dahlström, A. and Fuxe, K., 1965. Evidence for the existence of monoamine neurons in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neuron systems. **Acta Physiol. Scand. 64, suppl.** 247:1-36.

Dahlström, A. and Häggendal, J., 1966. Studies on the transport and life-span of amine storage granules in a peripheral adrenergic neuron system. **Acta Physiol. Scand.** 67:278-288.

Dahlström, A. and Häggendal, J., 1967. Studies on the transport and life-span of amine storage granules in the adrenergic neuron system of the rabbit sciatic nerve. **Acta Physiol. Scand.** 69:153-157.

Dahlström, A. and Jonason, J., 1968. Dopa-decarboxylase activity in sciatic nerves of the rat after constriction. **Europ. J. Pharmacol.** 4:377-383.

Dahlström, A., Jonason, J. and Norberg, K.-A., 1969. Monoamine oxidase activity in rat sciatic nerves after constriction.**Europ. J. Pharmacol.** 6:248-254.

Dahlström, A.B., Evans, C.A.N., Häggendal, C.J., Heiwall, P.-O. and Saunders, N.R., 1974. Rapid transport of acetylcholine in rat sciatic nerve proximal and distal to a lesion. **J. Neural Transmission** 35:1-11.

De Robertis, E. and Sotelo, J.R., 1952. Electron microscopy study of cultured nervous tissue. **Exp. Cell Res.** 3:433-452.

De Vito, J.L., Clausing, K.W. and Smith, O.A., 1974. Uptake and transport of horseradish peroxidase by cut end of the vagus nerve. **Brain Res.** 82:269-271.

Diamond, J. and Evans, C.A.N., 1960. Acetylcholine in regenerating motor nerves. **J. Physiol.** 154:69P.

Diepen, R. 1962. The difference between the neurosecretory pictures in various mammals. pp. 111-123 in Heller, H. and Clark, R.B. (Eds.). Neurosecretion. Academic Press, London.

Droz, B., 1965. Fate of newly synthesized proteins in neurons. In Leblond, C.P. and Warren, K.B. (Eds.). The Use of Radioautography in Investigating Protein Synthesis. Symp. Intl. Soc. Cell Biol. 4:159-174(175).

Academic Press, New York.

Droz, B. and Leblond, C.P., 1962. Migration of proteins along the axons of the sciatic nerve. **Science** 137:1047-1048.

Droz, B. and Leblond, C.P., 1963. Axonal migration of proteins in the central nervous system and peripheral nerves as shown by radioautography. **J. Comp. Neurol.** 121:325-346.

Droz, B. and Verne, J., 1960. Incorporation of S-35 marked methionine in the autonomic ganglionic cells in vivo and in vitro. **Acta Neuroveget.** (Vienna) 20:372-384.

Droz. B., Rambourg, A. and Koenig, H.L., 1975. The smooth endoplasmic reticulum: structure and role in the renewal of axonal membrane and synaptic vesicles by fast axonal transport. **Brain Res.** 93:1-13.

Duce, I.R. and Keen, P., 1976. A light and electron microscope study of changes occurring at the cut ends following section of the dorsal roots of rat spinal nerves. **Cell Tiss. Res.** 170:491-505.

Duce, I.R., Reeves, J.F. and Keen, P., 1976. A scanning electron microscope study of the development of free axonal sprouts at the cut ends of dorsal spinal nerve roots in the rat. **Cell Tiss. Res.** 170:507-513.

Edström, A. and Hanson, M., 1973a. Temperature effects on fast axonal transport of proteins in vitro in frog sciatic nerves. **Brain Res.** 58:345-354.

Edström, A. and Hanson, M., 1973b. Retrograde axonal transport of proteins in vitro in frog sciatic nerves. **Brain Res.** 61:311-320.

Engh, C.A., Schofield, B.H., Doty, S.B. and Robinson, R.A., 1971. Perikaryal synthetic function following reversible and irreversible peripheral axon injuries as shown by radioautography. **J. Comp. Neurol.** 142:465-480.

Estable, C., Acosta-Ferreira, W. and Sotelo, J.R., 1957. An electron microscope study of the regenerating nerve fibres. **Zeit. Zell. Mikr. Anat.** 46:387-399.

Evans, C.A.N. and Saunders, N.R., 1967. The distribution of acetylcholine in normal and in regenerating nerves. **J. Physiol.** 192:79-92.

Fabiato, A. and Fabiato, F., 1975. Effects of magnesium on contractile activation of skinned cardiac cells. **J. Physiol.** 249:497-517.

Fairbrother, R.W. and Hurst, E.W., 1930. The pathogenesis of, and propogation of the virus in, experimental poliomyelitis. J. Pathol. Bacteriol. 33:17-45.

Fillenz, M., Gagnon, C., Stoeckel, K. and Thoenen, H., 1976. Selective uptake and retrograde axonal transport of dopamine-ß-hydroxylase antibodies in peripheral adrenergic neurons. Brain Res. 114:293-303.

Forman, D.S., Padjen, A.L. and Siggins, G.R., 1975a. Effect of temperature on rapid retrograde axonal transport of optically detectable intra-axonal organelles. Neurosci. Abstr. 1:804.

Forman, D.S., Padjen, A.L. and Siggins, G.R., 1975b. Intra-axonal organelle movements as studied by cinematography and computer analysis. Exp. Brain Res. 23:S138.

Forman, D.S., Padjen, A.L. and Siggins, G.R., 1977a. Axonal transport of organelles visualized by light microscopy: cinemicrographic and computer analysis. Brain Res. 136:197-213.

Forman, D.S., Padjen, A.L. and Siggins, G.R., 1977b. Effect of temperature on the rapid retrograde transport of microscopically visible intra-axonal organelles. Brain Res. 136:215-226.

Francoeur, J. and Olszewski, J., 1968. Axonal reaction and axoplasmic flow as studied by radioautography. Neurol. 18:178-184.

Friede, R.L., 1959. Transport of oxidative enzymes in nerve fibers; a histochemical investigation of the regenerative cycle in neurons. Exp. Neurol. 1:441-466.

Friede, R.L., 1964a. Electrophoretic production of "reactive" axon swellings in vitro and their histochemical properties. Acta Neuropath. 3:217-228.

Friede, R.L., 1964b. Mechanisms involved in the production of reactive axonal swellings. J. Neuropath. Exp. Neurol. 23:164.

Friede, R.L. and Ho, K.-C., 1977. The relation of axonal transport of mitochondria with microtubules and other axoplasmic organelles. J. Physiol. 265:507-519.

Frizell, M. and Sjöstrand, J., 1974a. Retrograde axonal transport of rapidly migrating proteins in the vagus and hypoglossal nerves of the rabbit. J. Neurochem. 23:651-657.

Frizell, M. and Sjöstrand, J., 1974b. Transport of proteins, glycoproteins and cholinergic enzymes in regenerating hypoglossal neurons. J. **Neurochem.** 22:845-850.

Frizell, M. and Sjöstrand, J., 1974c. The axonal transport of slowly migrating [^{3}H] leucine labelled proteins and the regeneration rate in regenerating hypoglossal and vagus nerves of the rabbit. **Brain Res.** 81:267-283.

Frizell, M. and Sjöstrand, J., 1974d. The axonal transport of [^{3}H]fucose labelled glycoproteins in normal and regenerating peripheral nerves. **Brain Res.** 78:109-123.

Frizell, M., Hasselgren, P.O. and Sjöstrand, J., 1970. Axoplasmic transport of acetylcholinesterase and choline acetyltransferase in the vagus and hypoglossal nerve of the rabbit. **Exp. Brain Res.** 10:526-531.

Frizell, M., McLean, W.G. and Sjöstrand, J., 1975. Slow axonal transport of proteins; blockade by interruption of contact between cell body and axon. **Brain Res.** 86:67-73.

Frizell, M., McLean, W.G. and Sjöstrand, J., 1976. Retrograde axonal transport of rapidly migrating labelled proteins and glycoproteins in regenerating peripheral nerves. J. **Neurochem.** 27:191-196.

Gamache, F.W., Jr. and Gamache, J.F., 1974. Changes in axonal transport in neurones of **Asterias vulgaris** and **Asterias forbesei** produced by colchicine and dimethyl sulfoxide. **Cell Tiss. Res.** 152:423-435.

Geffen, L.B. and Ostberg, A., 1969. Distribution of granular vesicles in normal and constricted sympathetic neurones. J. **Physiol.** 204:583-592.

Geffen, L.B., Livett, B.G. and Rush, R.A., 1969. Immunological localization of chromogranins in sheep sympathetic neurones, and their release by nerve impulses. J. **Physiol.** 204:58P-59P.

Gerard, R.W., 1932. Nerve metabolism. **Physiol. Rev.** 12:469-592.

Gerard, R.W., 1950. Some aspects of neural growth, regeneration, and function. In Weiss, P. (Ed.). Genetic Neurology. pp. 199-207. University of Chicago Press, Chicago.

Goldberg, S. and Kotani, M., 1967. The projection of optic nerve fibres in the frog **Rana catesbiana** as studied by radioautography. Anat. Rec. 158:325-332.

Goldscheider, A., 1894a. Zur allgemeinen pathologie des nervensystems. I. Ueber die lehre von den trophischen centren. **Berlin. Klin. Wochenschr.** 31:421-425.

Goldscheider, A., 1894b. Zur allgemeinen pathologie des nervensystems. II. Ueber neuron-erkrankungen. **Berlin. Klin. Wochenschr.** 31:444-447.

Goodpasture, E.W., 1925a. A study of rabies, with reference to a neural transmission of the virus in rabbits, and the structure and significance of Negri bodies. **Amer. J. Pathol.** 1:547-582.

Goodpasture, E.W., 1925b. The axis-cylinders of peripheral nerves as portals of entry to the central nervous system for the virus of herpes simplex in experimentally infected rabbits. **Amer. J. Pathol.** 1:11-28.

Goodpasture, E.W. and Teague, O., 1923. Transmission of the virus of herpes febrilis along nerves in experimentally infected rabbits. **J. Med. Res.** 44:139-184.

Gould, R.P. and Holt, S.J., 1961. Observations on acid phosphatase and esterases in the rat sciatic nerve undergoing Wallerian degeneration. pp. 45-48 in Cytology of Nervous Tissue. Proceedings of the Anatomical Society of Great Britain and Ireland. Taylor and Francis, London.

Grafstein, B., 1967. Transport of protein by goldfish optic nerve fibers. **Science** 157:196-198.

Grafstein, B., 1975. The nerve cell body response to axotomy. **Exp. Neurol.** 48 (Number 3, Part 2):32-51.

Grafstein, B. and Murray, M., 1969. Transport of protein in goldfish optic nerve during regeneration. **Exp. Neurol.** 25:494-508.

Green, J., Erdmann, G. and Wellhöner, H.H., 1977. Is there retrograde axonal transport of tetanus toxin in both α and γ fibres? **Nature** 265:370.

Greenawalt, J.W., Rossi, C.S. and Lehninger, A.L., 1964. Effect of active accumuation of calcium and phosphate ions on the structure of rat liver mitochondria. J. Cell Biol. 23:21-38.

Gremo, F. and Marchisio, P.C., 1975a. Studies on the properties of axonal transport in retinal ganglion neurons of the chick embryo. **Exp. Brain Res.** 235:80 (abstr. no. 155).

Gremo, F. and Marchisio, P.C., 1975b. Dynamic properties of

axonal transport of proteins and glycoproteins: a study based on the effects of metaphase blocking drugs in the developing optic pathway of chick embryos. Cell Tiss. Res. 161:303-316.

Gremo, F., Sjöstrand, J. and Marchisio, P.C., 1974. Radioautographic analysis of ^{3}H-fucose labelled glycoproteins transported along the optic pathway of chick embryos. Cell Tiss. Res. 153:465-476.

Griffin, J.W., Drachman, D.B. and Price, D.L., 1976. Fast axonal transport in motor nerve regeneration. J. Neurobiol. 7:355-370.

Gutmann, E., Guttmann, L., Medawar, P.B. and Young, J.Z., 1942. The rate of regeneration of nerve. J. Exp. Biol. 19:14-44.

Halperin, J.J. and LaVail, J.H., 1975. A study of the dynamics of retrograde transport and accumulation of horseradish peroxidase in injured neurons. Brain Res. 100:253-269.

Hammond, G.R., 1977. The movement of optically detectable particles in intact myelinated axons. J. Physiol. 269:74P-75P.

Hammond, G.R. and Smith, R.S., 1977. Inhibition of the rapid movement of optically detectable axonal particles by colchicine and vinblastine. Brain Res. 128:227-242.

Hanson, M., Tonge, D. and Edström, A., 1975. Retrograde axonal transport of exogenous protein in frog nerves. Brain Res. 100:458-461.

Hay, E.D., 1960. The fine structure of nerves in the epidermis of regenerating salamander limbs. Exp. Cell Res. 19:299-317.

Hayden, K., Pomerat, C.M. and Smith, M., 1954. A note on the architecture of the mitochondrial cortex as seen in tissue cultures of nerve fibers. Texas Rep. Biol. Med. 12:470-473.

Hebb, C.O. and Silver, A., 1961. Gradient of choline acetylase activity. Nature 189:123-125.

Hebb, C.O. and Silver, A., 1963. The effect of transection on the level of choline acetylase in the goat's sciatic nerve. J. Physiol. 169:41P-42P.

Hebb, C.O. and Waites, G.M.H., 1956. Choline acetylase in antero- and retrograde degeneration of a cholinergic nerve. J. Physiol. 132:667-671.

Hendrickson, A.E. and Cowan, W.M. 1971. Changes in the rate of axoplasmic transport during postnatal development of the rabbit's optic nerve and tract. **Exp. Neurol.** 30:403-422.

Hendry, I.A., Stöckel, K., Thoenen, H. and Iversen, L.L., 1974. The retrograde axonal transport of nerve growth factor. **Brian Res.** 68:103-121.

Heslop, J.P., 1975. Axonal flow and fast transport in nerves. pp. 75-163 in Lowenstein, O. (Ed.). Advances in Comparative Physiology and Biochemistry, vol. 6. Academic Press, New York.

Heslop, J.P. and Howes, E.A., 1977. Saltatory movements of organelles in intact nerves of **Rhodnius prolixus Stål.** **J. Exp. Biol.** 70:247-257.

Hild, W., 1951. Experimentell-morphologische untersuchungen über das verhalten der 'neurosecretorischen bahn' nach hypophysenstieldurchtrennungen, eingriffen in den wasserhaushalt und belastung der osmoregulation. **Virchows Arch. Path. Anat.** 319:526-546.

Hild, W. and Zetler, G., 1953. Experimenteller beweis für die entstehung der sogenannten hypophysenhinterlappenwrikstoffe im hypothalamus. **Pflugers Arch.** 257:169-201.

Holton, P., 1960. pp. 192-194 in Polypeptides which Affect Smooth Muscles and Blood Vessels. Pergamon:London.

Holtzman, E. and Novikoff, A.B., 1965. Lysosomes in the rat sciatic nerve following crush. **J. Cell Biol.** 27:651-669.

Hughes, A., 1953. The growth of embryonic neurites. A study on cultures of chick neural tissues. **J. Anat.** 87:150-162.

Hunt, S.P. and Künzle, H., 1976. Bidirectional movement of label and transneuronal transport phenomena after injection of [^{3}H]adenosine into the central nervous system. **Brain Res.** 112:127-132.

Hutchinson, M.A., Koles, Z.J. and Smith, R.S., 1970. Conduction velocity in myelinated nerve fibres of **Xenopus laevis.** **J. Physiol.** 208:279-289.

Ingoglia, N.A., Sturman, J.A., Lindquist, T.D. and Gaull, G.E., 1976. Axonal migration of taurine in the goldfish visual system. **Brain Res.** 115:535-539.

Ingoglia, N.A., Weis, P. and Mycek, J., 1975. Axonal transport of RNA during regeneration of the optic nerves

of goldfish. J. Neurobiol. 6:549-563.

Inoue, S., 1960. Structural changes of nerve fibres in the early phases of limb regeneration in the adult newt with special references to fine structures of regenerating nerve fibres. Gunma J. Med. Sci. 9:302-328.

Iversen, L.L., Stöckel, K. and Thoenen, H., 1975. Autoradiographic studies of the retrograde axonal transport of nerve growth factor in mouse sympathetic neurones. Brain Res. 88:37-43.

Jeffrey, P.L. and Austin, L., 1973. Axoplasmic transport. Prog. Neurobiol. 2:205-255.

Johnson, G., Smith, R.S. and Lock, G.S.H., 1969. Accumulation of material at severed ends of myelinated nerve fibers. Amer. J. Physiol. 217:188-191.

Kapeller, K. and Mayor, D., 1966a. Ultrastructural changes proximal to a constriction in sympathetic axons during first 24 hours after operation. J. Anat. 100:439.

Kapeller, K. and Mayor, D., 1966b. Accumulation of noradrenaline proximal to the site of constriction of sympathetic nerves. J. Physiol. 182:44P.

Kapeller, K. and Mayor, D., 1966c. Effects of reserpine on the accumulation of noradrenaline in constricted sympathetic nerves. J. Physiol. 182:21P.

Kapeller, K. and Mayor, D., 1967a. The accumulation of noradrenaline in constricted sympathetic nerves as studied by fluorescence and electron microscopy. Proc. Roy. Soc. Lond. Ser. B 167:282-292.

Kapeller, K. and Mayor, D., 1967b. Ultrastructural appearances of degeneration and regeneration in constricted sympathetic nerves. J. Anat. 101:602.

Kapeller, K. and Mayor, D., 1967c. The accumulation of mitochondria proximal to a constriction in sympathetic nerves. J. Physiol. 191:70P.

Kapeller, K. and Mayor, D., 1969a. An electron microscopic study of the early changes distal to a constriction in sympathetic nerves. Proc. Roy. Soc. Lond. Ser. B 172:53-63.

Kapeller, K. and Mayor, D., 1969b. An electron microscopic study of the early changes proximal to a constriction in sympathetic nerves. Proc. Roy. Soc. Lond. Ser. B 172:39-51.

Karlsson, J.-O. and Sjöstrand, J., 1968. Transport of labelled proteins in the optic nerve and tract of the rabbit. **Brain Res.** 11:431-439.

Karlsson, J.-O. and Sjöstrand, J., 1971. Rapid intracellular transport of fucose-containing glycoproteins in retinal ganglion cells. **J. Neurochem.** 18:2209-2216.

Karlström, L. and Dahlström, A., 1973. The effect of different types of axonal trauma on the synthesis and transport of amine storage granules in rat sciatic nerves. **J. Neurobiol.** 4:191-200.

Kasa, P., 1968. Acetycholinesterase transport in the central and peripheral nervous tissue: the role of tubules in the enzyme transport. **Nature** 218:1265-1267.

Kerkut, G.A., Shapira, A. and Walker, R.J., 1967. The transport of ^{14}C-labelled material from CNS to muscle along a nerve trunk. **Comp. Bioch. Physiol.** 23:729-748.

Kim, C.C. and Strick, P.L., 1976. Critical factors involved in the demonstration of hoseradish peroxidase retrograde transport. **Brain Res.** 103:356-361.

Kirk, R.E., 1968. Experimental Design: Procedures for the Behavioral Sciences. Brooks/Cole, Belmont.

Kirkpatrick, J.B., 1971. Time-lapse cinematography of axoplasmic flow in peripheral nerves. Amer. Soc. for Cell Biol. Abstr. 11th Ann. Meeting. 11:340 (Abstr. no. 671).

Kirkpatrick, J.B. and Palmer, S.M., 1972. Ionic requirements for rapid particulate axoplasmic flow. Amer. J. Pathol. 66:4a (Abstr. no. 7).

Kirkpatrick, J.B. and Stern, L.Z., 1973. Axoplasmic flow in human sural nerve. Arch. Neurol. 28:308-312.

Kirkpatrick, J.B. and Stern, L.Z., 1975. Particulate components of axoplasmic flow. pp. 211-214 in Bradley, W.G., Gardner-Medwin, D. and Walton, J.B. (Eds.). Recent Advances in Myology. Proceedings of the Third International Congress on Muscle Diseases. Excerpta Medica, Amsterdam.

Kirkpatrick, J.B., Bray, J.J. and Palmer, S.M., 1972. Visualization of axoplasmic flow in vitro by Nomarski microscopy. Comparison to rapid flow of radioactive proteins. **Brain Res.** 43:1-10.

Koenig, H., 1958. The synthesis and peripheral flow of axoplasm. **Trans. Amer. Neurol. Assn.** 83:162-164.

Kreutzberg, G., 1963. Lokalisierter oxydoreduktasenanstieg bei der Wallerschen degeneration des peripheren nerves. **Naturwissenschaften** 50:96.

Kreutzberg, G.W., 1967. Autoradiographic study on incorporation of leucine-H^3 in peripheral nerves during regeneration. **Experientia** 23:33-34.

Kreutzberg, G.W. and Schubert, P., 1971a. Volume changes in the axon during regeneration. **Acta Neuropath.** 17:220-226.

Kreutzberg, G.W. and Schubert, P., 1971b. Changes in axonal flow during regeneration of mammalian motor nerves. **Acta Neuropath. suppl.** V:70-75.

Kreutzberg, G. and Wechsler, W., 1963. Histochemische untersuchungen oxydativer enzyme am regenerierenden nervus ischiadicus der ratte. **Acta Neuropath.** 2:349-361.

Kristensson, K., 1970a. Morphological studies of the neural spread of herpes simplex virus to the central nervous system. **Acta Neuropath.** 16:54-63.

Kristensson, K., 1970b. Transport of fluorescent protein tracer in peripheral nerves. **Acta Neuropath.** 16:293-300.

Kristensson, K., 1978. Retrograde transport of macromolecules in axons. **Ann. Rev. Pharmacol. Toxicol.** 18:97-110.

Kristensson, K. and Olsson, Y., 1971a. Uptake and retrograde axonal transport of peroxidase in hypoglossal neurones. **Acta Neuropath.** 19:1-9.

Kristensson, K. and Olsson, Y., 1971b. Uptake of exogenous proteins in mouse olfactory cells. **Acta Neuropath.** 19:145-154.

Kristensson, K. and Olsson, Y., 1971c. The perineurium as a diffusion barrier to protein tracers. Differences between mature and immature animals. **Acta Neuropath** 17:127-138.

Kristensson, K. and Olsson, Y., 1971d. Retrograde axonal transport of protein. **Brain Res.** 29:363-365.

Kirstensson, K. and Olsson, Y., 1973. Diffusion pathways and retrograde axonal transport of protein tracers in peripheral nerves. **Prog. Neurobiol.** 1:85-109.

Kristensson, K. and Olsson, Y., 1974. Retrograde transport of horeradish peroxidase in transected axons. 1. Time relationships between transport and induction of

chromatolysis. **Brain Res.** 79:101-109.

Kristensson, K. and Olsson, Y., 1975. Retrograde transport of horseradish peroxidase in transected axons. II. Relations between rate of transfer from the site of injury to the perikaryon and onset of chromatolysis. **J. Neurocytol.** 4:653-661.

Kristensson, K. and Olsson, Y., 1976. Retrograde transport of horseradish peroxidase in transected axons. 3. Entry into injured axons and subsequent localization in perikaryon. **Brain Res.** 115:201-213.

Kristensson, K. and Sjöstrand, J., 1972. Retrograde transport of protein tracer in the rabbit hypoglossal nerve during regeneration. **Brain Res.** 45:175-181.

Kristensson, K., Ghetti, B. and Wisniewski, H.M., 1974. Study on the propogation of **Herpes simplex** virus (type 2) into the brain after intraocular injection. **Brain Res.** 69:189-201.

Kristensson, K., Lycke, E. and Sjöstrand, J., 1971. Spread of herpes simplex virus in peripheral nerves. **Acta Neuropath.** 17:44-53.

Kristensson, K., Olsson, Y. and Sjöstrand, J., 1971. Axonal uptake and retrograde transport of exogenous proteins in the hypoglossal nerve. **Brain Res.** 32:399-406.

Laduron, P., 1970.Differential acumulation of various enzymes in constricted sciatic nerves. **Arch. Int. Pharmacodyn.** 185:200-203.

Laduron, P. and Belpaire, F., 1968. Transport of noradrenaline and dopamine-ß-hydroxylase in sympathetic nerves. **Life Sci.** 7:1-7.

Lajtha, A., 1961. Protein metabolism in nerve. pp. 268-275(276) in Folch-Pi, J. (Ed.). Chemical Pathology of the Nervous System. Proc. Third Intl. Neurochem. Symp. Pergamon Press, Oxford.

Lasek, R.J., 1966. Axoplasmic streaming in the cat dorsal root ganglion cell and the rat ventral motoneuron. **Anat. Rec.** 154:373-374.

Lasek, R.J., 1967. Bidirectional transport of radioactively labelled axoplasmic components. **Nature** 216:1212-1214.

Lasek, R., 1968a. Axoplasmic transport in cat dorsal root ganglion cells: as studied with (^{3}H)-L-leucine. **Brain Res.** 7:360-377.

Lasek, R., 1968b. Axoplasmic transport of labelled proteins in rat ventral motoneurons. Exp. Neurol. 21:41-51.

Lasek, R.J., 1970a. Axonal transport of proteins in dorsal root ganglion cells of the growing cat: A comparison of growing and mature neurons. Brain Res. 20:121-126.

Lasek, R.J., 1970b. Protein transport in neurons. Intl. Rev. Neurobiol. 13:289-324.

Lasek, R.J. and Hoffman, P.N., 1976. The neuronal cytoskeleton, axonal transport and axonal growth. pp. 1021-1049 in Goldman, R., Pollard, T. and Rosenbaum, J. (Eds.). Cell Motility, Book C, Microtubules and Related Proteins. Cold Spring Harbor Laboratory.

LaVail, J.H. and LaVail, M.M., 1972. Retrograde axonal transport in the central nervous system. Science 176:1416-1417.

LaVail, J.H. and LaVail, M.M., 1974. The retrograde intraaxonal transport of horseradish peroxidase in the chick visual system: a light and electron microscopic study. J. Comp. Neurol. 157:303-358.

Lee, J.C., 1963. Electron microscopy of Wallerian degeneration. J. Comp. Neurol. 120:65-79.

Leestma, J.E., 1976. Velocity measurements of particulate neuroplasmic flow in organized mammalian CNS tissue cultures. J. Neurobiol. 7:173-183.

Leestma, J.E. and Freeman, S.S., 1977. Computer-assisted analysis of particulate exoplasmic flow in organized CNS tissue cultures. J. Neurobiol. 8:453-467.

Lehninger, A.L., Carafoli, E. and Rossi, C.S., 1967. Energy-linked ion movements in mitochondrial systems. Adv. Enzymol. 29:259-320.

Lever, J.D., Spriggs, T.L.B., Graham, J.D.P. and Ivens, C., 1970. The distribution of ^{3}H-noradrenaline and acetylcholinesterase (AChE) proximal to constrictions of hypogastric and splenic nerves in the cat. J. Anat. 107:407-419.

Lewis, W.H., 1945. Axon growth and regeneration. Anat. Rec. 91:287.

Lewis, W.H., 1950. Motion picture of neurons and neuroglia in tissue culture. pp. 53-65 in Weiss, P. (Ed.). Genetic Neurology. University of Chicago Press, Chicago.

Litchy, W.J., 1973. Uptake and retrograde transport of

hoseradish peroxidase in frog sartorius nerve in vitro. **Brain Res.** 56:377-381.

Livett, B.G., Geffen, L.B. and Austin, L., 1968. Proximodistal transport of (^{14}C)-noradrenaline and protein in sympathetic nerves. **J. Neurochem.** 15:931-939.

Livett, B.G., Geffen, L.B. and Rush, R.A., 1969. Immunohistochemical evidence for the transport of dopamine-ß-hydroxylase and a catecholamine binding protein in sympathetic nerves. **Biochem. Pharmacol.** 18:923-924.

Lubińska, L., 1956. Outflow from cut ends of nerve fibres. **Exp. Cell Res.** 10:40-47.

Lubińska, L., 1959. Region of transition between preserved and regenerating parts of myelinated nerve fibers. **J. Comp. Neurol.** 113:315-335.

Lubińska, L., 1964. Axoplasmic streaming in regenerating and in normal nerve fibres. pp. 1-66(71) in Singer, M. and Schade, J.P. (Eds.). Progress in Brain Research, Volume 13. Mechanisms of Neural Regeneration. Elsevier, New York.

Lubińska, L., 1975. On axoplasmic flow. **Intl. Rev. Neurobiol.** 17:241-296.

Lubińska, L. and Niemierko, S., 1971. Velocity and intensity of bidirectional migration of acetylcholinesterase in transected nerves. **Brain Res.** 27:329-342.

Lubińska, L., Niemierko, S. and Oderfeld, B., 1961. Gradient of cholinesterase activity in nerve fibres. **Nature.** 189:122-123.

Lubińska, L., Niemierko, S., Oderfeld, B. and Szwarc, L., 1963. The distribution of acetylcholinesterase in peripheral nerves. **J. Neurochem.** 10:25-41.

Lubińska, L., Niemierko, S., Oderfeld-Nowak, B. and Szwarc, L., 1964. Behavior of acetylcholinesterase in isolated nerve segments. **J. Neurochem.** 11:493-503.

Lubińska, L., Niemierko, S., Oderfeld, B., Szwarc, L. and Zelená, J., 1963. Bidirectional movements of axoplasm in peripheral nerve fibres. **Acta Biol. Exp.** 23:239-247.

Lumsden, C.E., 1952. Quantitative studies on lipolytic enzyme activity in degenerating and regenerating nerve. **Quart. J. Exp. Physiol.** 37:45-57.

Marchisio, P.C. and Sjöstrand, J., 1972. Radioautographic

evidence for protein transport along the optic pathway of early chick embryos. J. Neurocytol. 1:101-108.

Marchisio, P.C., Gremo, F. and Sjöstrand, J., 1975. Axonal transport in embryonic neurons. The possibility of a proximo-distal axolemmal transfer of glycoproteins. Brain Res. 85:281-285.

Marchisio, P.C., Sjöstrand, J., Aglietta, M. and Karlsson, J.-O., 1973. The development of axonal transport of proteins and glycoproteins in the optic pathway of chick embryos. Brain Res. 63:273-284.

Marcum, M.J., Dedman, J.R., Brinkley, B.R. and Means, A.R., 1978. Control of microtubule assembly-disassembly by calcium-dependent regulator protein. Proc. Natl. Acad. Sci. USA 75:3771-3775.

Marinesco, G., 1924. Recherches histo-chimiques sur le rôle des ferments oxydants dans les phénomènes de la vie à l'état normal et pathologique. Ann. Anat. Pathol. Méd-Chir. 1:121-162.

Martinez, A.J. and Friede, R.L., 1970. Accumulation of axoplasmic organelles in swollen nerve fibers. Brain Res. 19:183-198.

Matsumoto, T., 1920. The granules, vacuoles and mitochondria in the sympathetic nerve-fibers cultivated in vitro. Bull. Johns Hopkins Hosp. 31:91-93.

Matthews, M.R., 1972. Evidence suggesting translocation of small dense-cored vesicles from the cell bodies to regenerating axon tips of adrenergic neurons. J. Anat. 111:508-510.

Matthews, M.R., 1973. An ultrastructural study of axonal changes following constriction of postganglionic branches of the superior cervical ganglion in the rat. Phil. Trans. Roy. Soc. Lond. Ser. B 264:479-505.

Matthews, M.R. and Raisman, G., 1972. A light and electron microscopic study of the cellular response to axonal injury in the superior cervical ganglion of the rat. Proc. Roy. Soc. Lond. Ser. B 181:43-79.

Mayor, D., Tomlinson, D.R., Banks, P. and Mraz, P., 1972. Microtubules and the intra-axonal transport of noradrenaline storage (dense-cored) vesicles. J. Anat. 111:344-345.

McEwen, B.S. and Grafstein, B., 1968. Fast and slow components in axonal transport of protein. J. Cell Biol. 38:494-508.

McEwen, B.S., Forman, D.S. and Grafstein, B., 1971. Components of fast and slow axonal transport in the goldfish optic nerve. J. Neurobiol. 2:361-377.

McLean, J.R. and Burnstock, G., 1972. Axoplasmic flow of adrenaline and monoamine oxidase in amphibian sympathetic nerves. Zeit. Zell. Mikr. Anat. 124:44-56.

McLean, W.G., Frizell, M. and Sjöstrand, J., 1976. Rapid axonal transport of labeled proteins in regenerating sensory and motor fibers of rabbit vagus nerve. Exp. Neurol. 52:242-249.

McMahan, U.J. and Kuffler, S.W., 1971. Visual identification of synaptic boutons on living ganglion cells and of varicosities in postganglionic axons in the heart of the frog. Proc. Roy. Soc. Lond.Ser. B 177:485-508.

Melamed, J. and Trujillo-Cenóz, O., 1963. Electron microscopic observations on reactional changes occurring in insect nerve fibres after transection. Zeit. Zell. Mikr. Anat. 59:851-856.

Meyer, H. and Ransom, F., 1904. Researches on tetanus.-Preliminary communication. Proc. Roy. Soc. Lond. 72:26-30.

Miani, N., 1960. Proximo-distal movement along the axon of protein synthesized in the perikaryon of regenerating neurons. Nature 185:541.

Miani, N., 1962. Evidence of a proximo-distal movement along the axon of phospholipid synthesized in the nerve-cell body. Nature 193:887-888.

Miani, N., 1963. Analysis of the somato-axonal movement of phospholipids in the vagus and hypoglossal nerves. J. Neurochem. 10:859-874.

Miani, N., 1964. Proximo-distal movement of phospholipid in the axoplasm of the intact and regenerating neurons. pp. 115-126 in Singer, M. and Schade, J.P. (Eds.). Progress in Brain Research, Volume 13. Mechanisms of Neural Regeneration. Elsevier. New York.

Morris, J.H., Hudson, A.R. and Weddell, G., 1972. A study of degeneration and regeneration in the divided rat sciatic nerve based on electron microscopy. III. Changes in the axons of the proximal stump. Zeit. Zell. Mikr. Anat. 124:131-164.

Nagatsu, I., Kondo, Y., Kato, T. and Nagatsu, T., 1976. Retrograde axoplasmic transport of inactive dopamine-ß -hydroxylase in sciatic nerves. Brain. Res. 116:277-285.

Nakai, J., 1956. Dissociated dorsal root ganglia in tissue culture. Amer. J. Anat. 99:81-129.

Navaratnam, V., Lewis, P.R. and Shute, C.C.D., 1968. Effects of vagotomy on the cholinesterase content of the preganglionic innervation of the rat heart. J. Anat. 103:225-232.

Neale, J.H. and Dimpfel, W., 1976. Movement of labeled macromolecules to the goldfish optic tectum following intraocular injection of ^{125}I-labeled tetanus toxin. Exp. Neurol. 53:355-362.

Nishida, E., 1978. Effects of solution variables on the calcium sensitivity of the microtubule assembly system. J. Biochem. 84:507-512.

Ochs, S., 1971a. Characteristics and a model for fast axoplasmic transport in nerve. J. Neurobiol. 2:331-345.

Ochs, S., 1971b. Local supply of energy to the fast axoplasmic transport mechanism. Proc. Natl. Acad. Sci. USA 68:1279-1282.

Ochs, S., 1972a. Fast transport of materials in mammalian nerve fibers. Science 176:252-260.

Ochs, S., 1972b. Fast axoplasmic transport of materials in mammalian nerve and its integrative role. Ann. N.Y. Acad. Sci. 193:43-57(58).

Ochs, S., 1975. Axoplasmic transport - a basis for neural pathology. pp. 213-230 in Dyck, P.J., Thomas, P.K. and Lambert, E.H. (Eds.). Peripheral Neuropathy. W.B. Saunders, Philadelphia.

Ochs, S., 1976. Fast axoplasmic transport in the fibres of chromatolyzed neurons. J. Physiol. 255:249-261.

Ochs, S. and Burger, E., 1958. Movement of substance proximo-distally in nerve axons as studied with spinal cord injections of radioactive phosphorus. Amer. J. Physiol. 194:499-506.

Ochs, S. and Johnson, J., 1969. Fast and slow phases of axoplasmic flow in ventral nerve root fibres. J. Neurochem. 16:845-853.

Ochs, S. and Ranish, N., 1969. Characteristics of the fast transport system in mammalian nerve fibers. J. Neurobiol. 1:247-261.

Ochs, S. and Ranish, N., 1970. Metabolic dependence of fast axoplasmic transport in nerve. Science 167:878-879.

Ochs, S. Dalrymple, D. and Richards, G., 1962. Axoplasmic flow in ventral root nerve fibers of the cat. Exp. Neurol. 5:349-363.

Ochs, S., Johnson, J. and Ng, M.-H., 1967. Protein incorporation and axoplasmic flow in motoneuron fibres following intra-cord injection of labelled leucine. J. Neurochem. 14:317-331.

Ochs, S., Kachmann, R. and DeMyer, W.E., 1960. Axoplasmic flow rates during nerve regeneration. Exp. Neurol. 2:627-637.

Ochs, S., Sabri, M.I. and Johnson, J., 1969. Fast transport system of materials in mammalian nerve fibres. Science 163:686-687.

Ochs, S., Worth, R.M. and Chan, S-Y., 1977. Calcium requirement for axoplasmic transport in mammalian nerve. Nature 270:748-750.

Parker, G.H., 1929a. The neurofibril hypothesis. Quart. Rev. Biol. 4:155-178.

Parker, G.H., 1929b. What are neurofibrils? Amer. Natur. 63:97-117.

Parker, G.H., 1932. On the trophic impulse so-called, its rate and nature. Amer. Natur. 66:147-158.

Parker, G.H. and Paine, V.L., 1934. Progressive nerve degeneration and its rate in the lateral-line nerve of the catfish. Amer. J. Anat. 54:1-25.

Partlow, L.M., Ross, D.C., Motwani, R. and McDougal, D.B. Jr., 1972. Transport of axonal enzymes in surviving segments of frog sciatic nerve. J. Genl. Physiol. 60:388-405.

Pellegrino de Iraldi, A. and de Robertis, E., 1968. The neurotubular system of the axon and the origin of granulated and non-granulated vesicles in regenerating nerves. Zeit. Zell. Mikr. Anat. 87:330-344.

Pellegrino de Iraldi, A. and de Robertis, E., 1970. Studies on the origin of the granulated and nongranulated vesicles. pp. 4-17 in Schumann, H.J. and Kroneberg, G. (Eds.). New Aspects of Storage and Release Mechanisms of Catecholamines. Springer-Verlag, New York.

Pellegrino de Iraldi, A. and Rodríguez de Lores Arnaiz, G., 1970. Thiamine pyrophosphatase activity in a fraction rich in Golgi-like structures from crushed sciatic nerve of the cat. J. Neurochem 17:1601-1606.

Pfeiffer, D.R., Kuo, T.H. and Tchen, T.T., 1976. Some effects of Ca^{2+}, Mg^{2+} and Mn^{2+} on the ultrastructure, light-scattering properties, and malic enzyme activity of adrenal cortex mitochondria. Arch. Biochem. Biophys. 176:556-563.

Pomerat, C.M., Hendelman, W.J., Raiborn, C.W. Jr. and Massey, J.F., 1967. Dynamic activities of nervous tissue in vitro. pp. 119-178 in Hydén, H. (Ed.). The Neuron. Elsevier, Amsterdam.

Price, D.L. and Porter, K.R., 1972. The response of ventral horn neurons to axonal transection. J. Cell Biol. 53:24-37.

Price, D.L., Griffin, J., Young, A., Peck, K. and Stocks, A., 1975. Tetanus toxin: direct evidence for retrograde intraaxonal transport. Science 188:945-947.

Prosser, C.L., 1973. Comparative Animal Physiology. Saunders, Philadelphia.

Rahmann, H., 1965. Zum stofftransport im zentralnervensystem der vertebraten. Zeit. Zell. Mikr. Anat. 66:878-890.

Rahmann, H., 1968. Syntheseort und ferntransport von proteinen im fischhirn. Zeit. Zell. Mikr. Anat. 86:214-237.

Rambourg, A. and Leblond, C.P., 1967. Electron microscope observations on the carbohydrate-rich cell coat present at the surface of cells in the rat. J. Cell Biol. 32:27-53.

Ramón y Cajal, S., 1928. Degeneration and Regeneration of the Nervous System. Translated by R.M. May. Oxford University Press, Cambridge.

Rebhun, L.I., 1972. Polarized intracellular particle transport: saltatory movements and cytoplasmic streaming. Intl. Rev. Cytol. 32:93-137.

Sabin, A.B., 1937. The nature and rate of centripetal progression of certain neurotropic viruses along peripheral nerves. Amer. J. Pathol. 13:615-616(617).

Sabin, A.B. and Olitsky, P.K., 1937. Pathological evidence of axonal and trans-synaptic progression of vesicular stomatitis and Eastern equine encephalomyelitis viruses. Amer. J. Pathol. 13:615.

Samorajski, T., 1957. Changes in phosphatase activity following transection of the sciatic nerve. J. Histochem. Cytochem. 5:15-27.

Samuels, A.J., Boyarski, L.L., Gerard, R.W., Libet, B. and Brust, M., 1951. Distribution, exchange and migration of phosphate compounds in the nervous system. **Amer. J. Physiol.** 164:1-15.

Sawyer, C.H., 1946. Cholinesterases in degenerating and regenerating peripheral nerves. **Amer. J. Physiol.** 146:246-253.

Sawyer, C.H. and Hollinshead, W.H., 1945. Cholinesterases in sympathetic fibres and ganglia. **J. Neurophysiol.** 8:137-153.

Sharrer, B., 1952. The effect of the interruption of the neurosecretory pathway in the insect, **Leucophaea maderae. Anat. Rec.** 112:386-387.

Scharrer, E. and Scharrer, B., 1954. Neurosecretion. pp. 953-1066 in Mollendorf, W. von and Bargmann, W. (Eds.). Handbuch der Mikroscopischen Anatomie des Menschen. Springer-Verlag, Berlin.

Scharrer, E.A. and Wittenstein, G.J., 1952. The effect of interruption of the hypothalamo-hypophysial neurosecretory pathway in the dog. **Anat. Rec.** 112:387.

Schlaepfer, W.W., 1974. Calcium-induced degeneration of axoplasm in isolated segments of rat peripheral nerve. **Brain Res.** 69:203-215.

Schlaepfer, W.W., 1977. Structural alterations of peripheral nerve induced by the calcium ionophore A23187. **Brain Res.** 136:1-9.

Schlaepfer, W.W. and Bunge, R.P., 1973. Effects of calcium ion concentration on the degeneration of amputated axons in tissue culture. **J. Cell Biol.** 59:456-470.

Schlote, W., 1964. Die läsionsbedingten primär-retrograden veränderungen der axone zentraler nervenfasern im elektronenmicroscopischen bild. **Acta Neuropath.** 4:138-157.

Schlote, W., 1966. Der aufbau von schichtenkörpern im axoplasma durchtrennter opticusfasern distal der läsion. **J. Ultrastr. Res.** 16:548-568.

Schlote, W. and Hager, H., 1960. Elektronenmikroskopische befunde zur feinstruktur von axonveränderungen im peritraumatischen bereich nach experimenteller strangdurchtrennung am rückenmark der weiszen ratte. **Naturwissenschaften** 47:448-451.

Schwab, M.E. and Thoenen, H., 1977. Retrograde axonal and

transsynaptic transport of macromolecules: physiological and pathophysiological importance. Agnts. Actns. 7:361-368.

Schwartz, J.H., 1979. Axonal transport: components, mechanisms, and specificity. Ann. Rev. Neurosci. 2:467-504.

Schwarzacher, H.G., 1958. Der cholinesterasegehalt motorischer nervenzellen während der axonalen reaktion. Acta Anat. 32:51-65.

Scott, F.H., 1905. On the metabolism and action of nerve cells. Brain 28:506-526.

Scott, F.H., 1906. On the relation of nerve cells to fatigue of their nerve fibres. J. Physiol. 34:145-162.

Sjöstrand, J., 1970. Fast and slow components of axoplasmic transport in the hypoglossal and vagus nerves of the rabbit. Brain Res. 18:461-467.

Sjöstrand, J. and Frizell, M., 1975. Retrograde axonal transport of rapidly migrating proteins in peripheral nerves. Brain Res. 85:325-330.

Sjöstrand, J., Frizell, M. and Hasselgren, P.-O., 1970. Effects of colchicine on axonal transport in peripheral nerves. J. Neurochem. 17:1563-1570.

Sjöstrand, J., Karlsson, J.-O. and Marchisio, P.C., 1973. Axonal transport in growing and mature retinal ganglion cells. Brain Res. 62:395-397.

Skangiel-Kramska, J., Niemierko, S. and Lubińska, L., 1969. Comparison of the behaviour of a soluble and a membrane-bound enzyme in transected peripheral nerves. J. Neurochem. 16:921-926.

Smith, R.S., 1971. Centripetal movement of particles in myelinated axons. Cytobios 3:259-262.

Smith, R.S., 1972a. Detection of organelles in myelinated nerve fibers by dark-field microscopy. Can. J. Physiol. Pharmacol. 50:467-469.

Smith, R.S., 1972b. Particle movement in myelinated axons. Physiol. Can. 3:33.

Smith, R.S., 1972c. Moving organelles in adult myelinated nerve: attempted identification. Proc. Cdn. Fed. Biol. Soc. 15:Abstr. no. 735.

Smith, R.S., 1973. Microtubule and neurofilament densities

in amphibian spinal root nerve fibers: relationship to axoplasmic transport. Can. J. Physiol. Pharmacol. 51:798-806.

Smith, R.S., 1978. Morphological characterization of organelles undergoing rapid retrograde transport in isolated axons. IVth Intnatl. Congr. on Neuromusc. Diseases. Abstr. 354.

Smith, R.S., 1980. The short term accumulation of axonally transported organelles in the region of localized lesions of single myelinated axons. J. Neurocytol. (In Press).

Smith, R.S. and Cooper, P.D., 1979. Velocity variability and temperature dependence of retrograde particle transport in amphibian axons. Proc. Cdn. Fed. Biol. Soc. 22:110(Abstr. no. 437).

Smith, R.S. and Koles, Z.J., 1976. Mean velocity of optically detected intraaxonal particles measured by a cross-correlation method. Can. J. Physiol. Pharmacol. 54:859-869.

Smith, R.S. and Lännergren, J., 1968. Types of motor units in the skeletal muscle of Xenopus laevis. Nature 217:281-283.

Smith, R.S. and Snyder, R.E., 1979. A multiwire proportional chamber study of axoplasmic transport in frog sciatic nerve involving interruption of somatic supply. Brain Res. 161:237-251.

Smith, R.S., Koles, Z.J. and McLeod, K.D., 1979. Analysis of particle motion in axons. Can Physiol. 10:192.

Sokal, R.R. and Rohlf, F.J., 1969. Biometry. The Principles and Practice of Statistics in Biological Research. W.H. Freeman, San Francisco.

Sotelo, C. and Taxi, J., 1971. Axonal flow of catecholamines in constricted sciatic nerves of the rat. An autoradiographic study. Anat. Rec. 169:435.

Sotelo, C. and Taxi, J., 1973. On the axonal migration of catecholamines in constricted sciatic nerve of the rat. A radioautographic study. Zeit. Zell. Mikr. Anat. 138:345-370.

Speidel, C.C., 1932. Studies of living nerves. I. The movements of individual sheath cells and nerve sprouts correlated with the process of myelin-sheath formation in amphibian larvae. J. Exp. Zool. 61:279-317.

Speidel, C.C., 1933. Studies of living nerves. II. Activities of ameboid growth cones, sheath cells, and myelin segments, as revealed by prolonged observation of individual nerve fibres in frog tadpoles. **Amer. J. Anat.** 52:1-79.

Speidel, C.C., 1935a. Studies on living nerves. III. Phenomena of nerve irritation, recovery, degeneration and repair. **J. Comp. Neurol.** 61:1-82.

Speidel, C.C., 1935b. Studies on living nerves. IV. Growth, regeneration, and myelination of the peripheral nerves in salamanders. **Biol. Bull.** 68:140-163.

Spencer, P.S., 1972. Reappraisal of the model for "bulk axoplasmic flow". **Nature New Biol.** 240:283-285.

Stöckel, K., Paravicini, U. and Thoenen, H., 1974. Specificity of the retrograde axonal transport of nerve growth factor. **Brain Res.** 76:413-421.

Stöckel, K., Schwab, M. and Thoenen, H., 1975. Comparison between the retrograde axonal transport of nerve growth factor and tetanus toxin in motor, sensory and adrnegic neurons. **Brain Res.** 99:1-16.

Stoeckel, K., Schwab, M. and Thoenen, H., 1975. Specificity of retrograde transport of nerve growth factor (NGF) in sensory neurons: a biochemical and morphological study. **Brain Res.** 89:1-14.

Stoeckel, M.E., Hindelang-Gertner, C., Dellmann, H.-D., Porte, A. and Stutinsky, F., 1975. Subcellular localization of calcium in the mouse hypophysis I. Calcium distribution in the adeno- and neurohypophysis under normal conditions. **Cell Tiss. Res.** 157:307-322.

Taylor, A.C. and Weiss, P., 1965. Demonstration of axonal flow by the movement of tritium-labelled protein in mature optic nerve fibres. **Proc. Natl. Acad. Sci. USA** 54:1521-1527.

Teale, F.H. and Embleton, D., 1919. Studies in infection.-II. The paths of spread of bacterial exotoxins with special reference to tetanus toxin. **J. Pathol. Bacteriol.** 23:50-68.

Thiele, F.H. and Embleton, D., 1914. Infection: paths of spread in bacterial infection. **Proc. Roy. Soc. Med.** 7 (Pathol. Sectn.):69-96.

Thomsen, E., 1954. Experimental evidence for the transport of secretory material in the axons of the neurosecretory cells of **Calliphora erythrocephala**. MEIG. Publ. Stazione

Zool. (Napoli), Suppl. 24:48-49.

Tomlinson, D.R., Till, R.J.L. and Mayor, D., 1975. The effect of tetrabenazine on the accumulation of noradrenaline in constricted postganglionic sympathetic nerves in vitro. Cell Tiss. Res. 160:495-504.

Townes-Anderson, E. and Raviola, G., 1978. Degeneration and regeneration of autonomic nerve endings in the anterior part of rhesus monkey ciliary muscle. J. Neurocytol. 7:583-600.

Van Breemen, V.L., Anderson, E. and Reger, J.F., 1958. An attempt to determine the origin of synaptic vesicles. Exp. Cell Res., Suppl. 5:153-167.

Verne, J. and Droz, B., 1960. Migration of radioactivity in the superior cervical ganglion after injection of S35 labelled methionine. Experientia 16:77-78.

Waelsch, H., 1958. Some aspects of amino acid and protein metabolism of the nervous system. J. Nerv. Ment. Dis. 126:33-39.

Wallace, B.J., Volk. B.W. and Lazarus, S.S., 1964. Fine structural localization of acid phosphatase activity in neurons of Tay-Sachs disease. J. Neuropath. Exp. Neurol. 23:676-691.

Watson, W.E., 1968a. Observations on the nucleolar and total cell body nucleic acid of injured nerve cells. J. Physiol. 196:655-676.

Watson, W.E., 1968b. Centripetal passage of labelled molecules along mammalian motor axons. J. Physiol. 196:122 P.

Watson, W.E., 1974. Cellular responses to axotomy and to related procedures. Brit. Med. Bull. 30:112-115.

Webster, H. de F., 1962. Transient, focal accumulation of axonal mitochondria during the early stages of Wallerian degeneration. J. Cell Biol. 12:361-383.

Wechsler, W. and Hager, H., 1962. Elektronenmikroskopische befunde zer feinstruktur von axonveränderungen in regenerierenden nervenfasern des nervus ischiadicus der weiszen ratte. Acta Neuropath. 1:489-506.

Weisenberg, R.C. and Deery, W.J., 1976. Role of nucleotide hydrolysis in microtubule assembly. Nature 263:792-793.

Weiss, P., 1944. Evidence of perpetual proximo-distal growth of nerve fibers. Biol. Bull. 87:160.

Weiss, P. (Ed.), 1950. Genetic Neurology. University of Chicago Press, Chicago.

Weiss, P., 1961. The concept of perpetual neuronal growth and proximo-distal substance convection. pp. 220-242 in Kety, S.S. and Elkes, J. (Eds.). Regional Neurochemistry. The Regional Chemistry, Physiology and Pharmacology of the Nervous System. Proc. Fourth Intl. Neurochem. Symp. Pegamon Press, Oxford.

Weiss, P., 1967a. Neuronal dynamics and axonal flow, III. Cellulifugal transport of labeled neuroplasm in isolated nerve preparations. Proc. Natl. Acad. Sci. USA 57:1239-1245.

Weiss, P., 1967b. Neuronal dynamics, an essay by P. Weiss. Neurosci. Res. Progm. Bull. 5:371-400.

Weiss, P.A., 1969a. Neuronal dynamics and neuroplasmic ("axonal") flow. In Barondes, S.H. (Ed.). Cellular Dynamics of the Neuron. Symp. Intl. Soc. Cell Biol. v. 8 pp. 3-34.

Weiss, P., 1969b. Neuronal dynamics. Neurosci. Res. Symp. Summ. 3:255-284.

Weiss, P.A., 1970. Neuronal dynamics and neuroplasmic flow. pp. 840-850 in Schmitt, F.O. (Ed.). The Neurosciences. Second Study Program. Rockefeller University Press, New York.

Weiss, P.A., 1972. Neuronal dynamics and axonal flow, V. The semisolid state of the moving axonal column. Proc. Natl. Acad. Sci. USA 69:620-623.

Weiss, P. and Davis, H., 1943. Pressure block in nerves provided with arterial sleeves. J. Neurophysiol. 6:269-286.

Weiss, P. and Hiscoe, H.B., 1948. Experiments on the mechanism of nerve growth. J. Exp. Zool. 107:315-395.

Weiss, P. and Holland, Y., 1967. Neuronal dynamics and axonal flow. II. The olefactory nerve as model test object. Proc. Natl. Acad. Sci. USA 57:258-264.

Weiss, P. and Pillai, A., 1965. Convection and fate of mitochondria in nerve fibers: axonal flow as vehicle. Proc. Natl. Acad. Sci. USA 54:48-56.

Weiss, P. and Taylor, A.C., 1944. Impairment of growth and myelinization in regenerating nerve fibers subject to constriction. Proc. Soc. Exp. Biol. Med. 55:77-80.

*Weiss, P., Taylor, A.C. and Pillai, P.A., 1962. The nerve fibre as a system in continuous flow: microcinematographic and electronmicroscopic demonstrations. Science 136:330.

Wellhöner, H.H., Erdmann, G. and Wiegand, H., 1976. Neural Transport of Tetanus Toxin. In Technical Comments. Science 192:158.

Wettstein, R. and Sotelo, J.R., 1963. Electron microscope study on the regenerative process of peripheral nerves of mice. Zeit. Zell. Mikr. Anat. 59:708-730.

Winkelmann, R.K. and Schmit, R.W. 1957. A simple silver method for nerve axoplasm. Mayo Clin. Proc. 32:217-222.

Wise, S.P. and Jones, E.G., 1976. Transneuronal or retrograde transport of [^{3}H] adenosine in the rat somatic sensory system. Brain Res. 107:127-131.

Young, J.Z., 1942. The functional repair of nervous tissue. Physiol. Rev. 22:318-374.

Young, J.Z., 1945. Structure, degeneration and repair of nerve fibres. Nature 156:132-136.

Young, R.W. and Droz, B., 1968. The renewal of protein in retinal rods and cones. J. Cell Biol. 39:169-184.

Yuien, K., 1928. On the direction of the lymphatic stream in the nerve. Fol. Anat. Jap. 6:301-309.

Zacks, S.I. and Saito, A., 1969. Uptake of exogenous horseradish peroxidase by coated vesicles in mouse neuromuscular junctions. J. Histochem. Cytochem. 17:161-170.

Zacks, S.I. and Sheff, M.F., 1970. Tetanism: pathobiological aspects of the action of tetanal toxin in the nervous system and skeletal muscle. Neurosci. Res. 3:209-287.

Zelená, J., 1968. Bidirectional movements of mitochondria along axons of an isolated nerve segment. Zeit. Zell. Mikr. Anat. 92:186-196.

Zelená, J., 1969. Bidirectional shift of mitochondria in axons after injury. pp. 73-94 in Barondes, S.H. (Ed.). Cellular Dynamics of the Neuron. Academic Press, New York.

Zelená, J. and Lubińska, L., 1962. Early changes of acetylcholinesterase activity near the lesion in crushed nerves. Physiol. Bohemoslov. 11:261-268.

Zelená, J., Lubińska, L. and Gutmann, E., 1968.
Accumulations of organelles at the ends of interrupted axons. Zeit. Zell. Mikr. Anat. 91:200-219.